METHODS AND COMPOSITIONS OF ECDYSOZOAN MOLT INHIBITION

Statement as to Federally Sponsored Research

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Background of the Invention

In general, the invention features methods and compositions that disrupt molting and are therefore useful targets for pesticides.

Nematodes represent one out of every five animals on the planet, and virtually all plant and animal species are targeted by at least one parasitic nematode. Plant-parasitic nematodes reduce the yield of the world's 40 major food staples resulting in losses of approximately 12.3% annually. Parasitic nematodes also damage human and domestic animal health. Lymphatic filariasis and elephantiasis are among the most devastating human tropical diseases. The World Health Organization estimated that these diseases affected 120 million people worldwide in 1992.

The impact of nematodes on human, animal, and plant health has resulted in the search for effective nematicides. Benzimidazoles and avermectins are two common nematicides, which target microtubule assembly and muscle activity, respectively. Unfortunately, resistance to these compounds is increasingly common. In addition, these compounds can have toxic effects on humans and other animals. Moreover, these nematicides are not effective against all nematodes. Thus more effective and specific nematicides are required.

Summary of the Invention

The present invention features improved methods and compositions for inhibiting molting in Ecdysozoans, including nematodes, parasitic nematodes, and insects.

In one aspect, the invention provides a method for identifying a candidate compound that disrupts molting in an Ecdysozoan (e.g., an insect or nematode). The method includes the steps of: (a) providing a cell expressing a *mlt* nucleic acid molecule or an ortholog of a *mlt* nucleic acid molecule; (b) contacting the cell with a candidate compound; and (c) comparing the expression of the *mlt* nucleic acid molecule in the cell contacted with the candidate compound with the expression of the nucleic acid molecule in a control cell not contacted with said candidate compound, where an alteration in expression identifies the candidate compound as a candidate compound that disrupts molting.

In a related aspect, the invention provides another method for identifying a candidate compound that disrupts molting in a nematode. The method includes the steps of: (a) providing a nematode cell expressing a *mlt* nucleic acid molecule; (b) contacting the nematode cell with a candidate compound; and (c) comparing the expression of the *mlt* nucleic acid molecule in the cell contacted with the candidate compound with the expression of the nucleic acid molecule in a control cell not contacted with said candidate compound, where an alteration in expression identifies the candidate compound as a candidate compound that modulates molting.

In various embodiments of the previous aspects, the method identifies a compound that increases or decreases transcription of a *mlt* nucleic acid molecule. In other embodiments of the previous aspects, the method identifies a compound that increases or decreases translation of an mRNA transcribed from the *mlt* nucleic acid molecule. In still other embodiments of the identification methods described herein, the compound is a member of a chemical library. In preferred embodiments, the cell is in a nematode.

Typically, a compound that decreases transcription or translation of a *mlt* nucleic acid molecule is useful in the invention. For some applications, however, a compound that increases transcription or translation of a *mlt* nucleic acid molecule is useful, for example, a *mlt* nucleic acid (e.g.,W08F4.6, F09B12.1, or W01F3.3) that when overexpressed leads to larval arrest or death, or a *mlt* nucleic acid (e.g., C17G1.6, CD4.6, C42D8.5, F08C6.1) that encodes a secreted protease, which degrades Ecdysozoan cuticle and leads to larval arrest or death.

In a related aspect, the invention provides yet another method for identifying a candidate compound that disrupts molting in an Ecdysozoan. The method involves (a) providing a cell expressing a MLT polypeptide; (b) contacting the cell with a candidate compound; and (c) comparing the biological activity of the MLT polypeptide in the cell contacted with the candidate compound to a control cell not contacted with said candidate compound, where an alteration in the biological activity of the MLT polypeptide identifies the candidate compound as a candidate compound that disrupts molting.

In various embodiments, the cell is a nematode cell or a mammalian cell. In other embodiments, the MLT polypeptide is a protease. In still other embodiments, the biological activity of MLT polypeptide is monitored with an enzymatic assay or an immunological assay. In other preferred embodiments, the cell is in a nematode and the biological activity is monitored by detecting molting.

In another related aspect, the invention provides yet another method for identifying a candidate compound that disrupts molting. The method includes the steps of: (a) contacting a nematode with a candidate compound; and (b) comparing molting in the nematode contacted with the candidate compound to a control nematode not contacted with said candidate compound, where an alteration in molting identifies the candidate compound as a candidate compound that disrupts molting.

In yet another related aspect, the invention provides a yet further method of identifying a candidate compound that disrupts Ecdysozoan molting. The method includes the steps of: (a) contacting a cell containing a *mlt* nucleic acid regulatory region fused to a detectable reporter gene with a candidate compound; (b) detecting the expression of the reporter gene; and (c) comparing the reporter gene expression in the cell contacted with the candidate compound with a control cell not contacted with the candidate compound, where an alteration in the expression of the reporter gene identifies the candidate compound as a candidate compound that disrupts molting.

In various embodiments of the previous aspect, the alteration is an alteration in the timing of reporter gene expression of at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99% relative to the timing of expression in a control nematode not contacted with the candidate compound. In another embodiment, the alteration is an alteration in the level of expression of the reporter gene of at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99% relative to the level of expression in a control nematode not contacted with the candidate compound. In another embodiment, the alteration is an alteration in the cellular expression pattern of the reporter gene relative to the cellular expression pattern in a control nematode not contacted with the candidate compound.

In another related aspect, the invention provides a method for identifying a candidate compound that disrupts Ecdysozoan molting. The method includes the steps of: (a) contacting a MLT polypeptide with a candidate compound; and (b) detecting binding of said candidate compound to said MLT polypeptide, wherein said binding identifies said candidate compound as a candidate compound that disrupts molting.

In other aspects, the invention generally features an isolated RNA *mlt* nucleic acid inhibitor comprising at least a portion of a naturally occurring *mlt* nucleic acid molecule of an organism, or its complement, where the *mlt* nucleic acid is selected from the group consisting of any or all of the following

B0024.14, C09G5.6, C11H1.3, C23F12.1, B0272.5, C34G6.6, C37C3.3, C42D8.5, CD4.4, CD4.6, D1054.15, F08C6.1, F09B12.1, F16H9.2, F18A1.3, F20G4.1, F25B4.6, F33A8.1, F33C8.3, F38H4.9, F40G9.1, F41C3.4, F41H10.7, F45G2.5, F49C12.12, F52B11.3, F53B8.1, F54A5.1, F54C9.2, F57B9.2, H04M03.4, H19M22.1, K05C4.1, K06B4.5, K07C5.6, K07D8.1, K08B4.1, K09H9.6, M03F4.7, M03F8.3, M162.6, M6.1, M88.6, R05D11.3, R07E4.6, R11G11.1, T01C3.1, T01H3.1, T14F9.1, T19B10.2, T23F2.1, T24H7.2, W01F3.3, W08F4.6, W09B6.1, W10G6.3, Y111B2A.14, Y37D8A.10, Y38F2AL.3, Y48B6A.3, ZC101.2, ZK1073.1, ZK1151.1, ZK262.8, ZK430.8, ZK686.3, ZK783.1, ZK970.4, C09F12.1, C09H10.2, C17H12.14, C37C3.2, D2085.1, EEED8.5, F10E9.7, F19F10.9, F28F8.5, F32D1.2, F35H10.4, F41E7.1, F42A8.1, F54B3.3, F55A3.3, F56F3.5, H06I04.4a, K06A4.6, K10D6.1, R06A10.1, T07D10.1, Y17G7A.2, Y38F2AL.3, Y41D4B.21, Y41D4B.5, Y45F10B.5, Y55H10A.1, ZK1236.3, ZK265.5, ZK265.6, ZK652.1, Y54E10BR.5, B0513.1, R06A4.9, Y105E8B.1, Y47D3B.1, Y54F10AL.2, T17H7.3, H27M09.5, F45E10.2, F25H8.6, K04A8.6, ZC13.3, T19A5.3, F32D8.6, F53F4.3, F56C9.12, T25B9.10, ZK154.3, Y37D8A.19, Y37D8A.21, Y71F9AL.7, Y51H1A.3, W03F9.10, ZK945.2, ZK637.4, C30F8.2, F32H2.9, Y87G2A.5, Y53F4B.22, Y77E11A.13, C15H11.7, Y113G7B.23, C53H9.1, W09C5.6, T24B8.1, Y71A12B.1, C26C6.3, C42D8.5, F53G12.3, Y41D4B.10, and F10C1.5, or an ortholog of any or all of these *mlt* nucleic acid molecules, where the RNA *mlt* nucleic acid inhibitor comprises at least a portion of a naturally occurring *mlt* nucleic acid inhibitor, or is capable of hybridizing to a naturally occurring *mlt* nucleic acid molecule, and decreases expression from a naturally occurring *mlt* nucleic acid molecule in the organism. In some embodiments, the naturally occurring *mlt* nucleic acid had been previously identified as functioning in molting, but had not been identified as the target for a nematicide, insecticide, or other compound that inhibits molting (e.g., C01H6.5, C17G1.6; C45B2.7, F11C1.6, F18C12.2, F29D11.1, F53G12.3, F56C11.1, K04F10.4, T05C12.10, T27F2.1,

Y23H5A.7, and ZK270.1). In other embodiments, the naturally occurring *mlt* nucleic acid encodes a component of a secretory pathway (e.g., ZK1014.1,H15N14.1, F26H9.6, Y63D3A.5, C56C10.3, ZK180.4, F57H12.1, C39F7.4, Y113G7A.3, R160.1, C02C6.1, E03H4.8, F59E10.3, K12H4.4, D1014.3, C13B9.3, F43D9.3). In other embodiments, the naturally occurring *mlt* nucleic acid encodes a protein that functions in protein synthesis (e.g., B0336.10, B0393.1, C04F12.4, C23G10.3, D1007.6, F28D1.7, F35H10.4, F37C12.11, F37C12.9, F40F11.1, F53A3.3, T01C3.6, T05F1.3, Y45F10D.12). In still other embodiments, the inactivation or inhibition of a naturally occurring *mlt* nucleic acid produces *mlt* defects in less than 5% of larvae (e.g., C09F12.1, C09H10.2, C17H12.14, C37C3.2, C37C3.3, D2085.1, EEED8.5, F10E9.7, F19F10.9, F28F8.5, F32D1.2, F35H10.4, F41E7.1, F42A8.1, F54B3.3, F55A3.3, F56F3.5, H06I04.4a, K06A4.6, K10D6.1, R06A10.1, T07D10.1, Y17G7A.2, Y23H5A.7, Y38F2AL.3, Y41D4B.21, Y41D4B.5, Y41D4B.5, Y45F10B.5, Y55H10A.1, ZK1236.3, ZK265.5, ZK265.6, ZK652.1).

In preferred embodiments, the naturally occurring *mlt* nucleic acid molecule is an ortholog of a *mlt* nucleic acid molecule. The ortholog is selected from the group consisting of any one or all of the following M90806, NM_134578, AY075331, BG310588, BE758466, BG227161, BM346811, BG226227, BF169279, BE580288, BG893621, BQ625515, BI746672, AA471404, BE579677, BI500192, BI782938, BI073876, BF060055, AI723670, BI746256, BM882137, BM277122, BM880769, BI501765, BE581131, AI539970, BE580231, BE238916, AY060635, NM_143476, AC008339, L02793, NM_079167, J02727, NM_139674, NM_079763, NM_057268, NM_137449, NM_079419, NM_080092, AAF51201, NM_057698, NM_080132, NM_132335, AJ487018, NM_080072, AY094832, NM_057520, NM_136653, NM_078644, AY075331, M90806, NM_079419, NM_080092, AAF51201, NM_057698, NM_134578, AY071265, AY060235, NM_078577, NM_057621, AY089504, NM_135238, X78577, AY118647,

NM_140652, AY113364, NM_079972, X58374, NM_132550, AY052122 AY060893, AY058709 AA161577, CAAC01000031, CAAC01000016, BI744615, BG224680, AW114337, BM281377, BU585500, BG577863, BQ091075, AW257707, BF014893, BQ613344, CAAC01000088, BG735742, CAAC01000028, AA110597, BI863834, AI987143, BI782814, BI744849, and BG735807.

In other preferred embodiments, the naturally occurring *mlt* nucleic acid molecule is a Drosophila ortholog of a *mlt* nucleic acid molecule. The Drosophila ortholog is selected from the group consisting of any one or all of the following ref|NM_079167, gb|M90806, ref|NM_079419, ref|NM_080092, gb| AY075331, ref| NM_057698, ref|NM_132335, ref| NM_134871, gb|AAF51201, ref|NM_136653, ref|NM_057520, ref| NM_080132, gb|AY094832, emb| AJ487018, ref|NM_080072, emb| AJ011925, ref|NM_078644, ref|NM_132550, ref|NM_079972, gb|AY089504, emb|X78577, gb|AY118647, gb| AY071265, ref|NM_140652, ref|NM_078577, emb|X58374, ref|NM_134578, gb|AY058709, gb|AY060235, gb|AY052122, AY060893, gb|AY113364, ref|NM_135238, ref|NM_057621, ref|NM_136498, ref| NM_143476, ref|NM_137449, gb|M16152, ref|NM_057268, ref|NM_139674, gb|L02793, gb|AY060635, gb|AC008339.

In other preferred embodiments of the previous aspects, the RNA *mlt* nucleic acid inhibitor is a double stranded RNA molecule that decreases expression in the organism by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99% from a naturally occurring *mlt* nucleic acid molecule. In other preferred embodiments, the RNA *mlt* nucleic acid inhibitor is an antisense RNA molecule that is complementary to at least six, seven, eight, nine, ten, fifteen, twenty, twenty-five, thirty, forty, fifty, seventy-five, or one hundred nucleotides of the *mlt* nucleic acid molecule and decreases expression in the organism by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99% from a nucleic acid molecule to which it is complementary. In other preferred embodiments, the RNA *mlt* nucleic acid

inhibitor is an siRNA molecule that comprises at least fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, or twenty-six nucleic acids of a *mlt* nucleic acid molecule and decreases expression in said organism by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In related aspects, the invention features a vector comprising a *mlt* nucleic acid that encodes a MLT polypeptide or a nucleic acid encoding an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), positioned for expression, and a host cell (e.g., plant, animal, or bacterial cell) containing the vector. For some applications, the vector used is a vector described in Fraser et al. (*Nature*, 408:325-30, 2000), hereby incorporated by reference.

In another aspect, the invention provides a method for reducing or ameliorating a parasitic nematode infection in an organism (e.g., a human or domestic mammal, such as a cow, sheep, goat, pig, horse, dog, or cat). The method includes contacting the organism with a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA).

In a related aspect, the invention provides a method for reducing or ameliorating a parasitic nematode infection in an organism (e.g., a human or domestic mammal, such as a cow, sheep, goat, pig, horse, dog, or cat). The method includes contacting the organism with a MLT polypeptide.

In other related aspects, the invention provides a pharmaceutical composition including a MLT polypeptide or portion thereof, encoded by a *mlt* nucleic acid or an ortholog of the nucleic acid molecule, and a pharmaceutical excipient, that ameliorates a parasite infection in an animal.

In other related aspects, the invention provides a pharmaceutical composition including a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), or portion thereof, and a pharmaceutical excipient, which ameliorates a parasite infection in an animal.

In another aspect, the invention provides a method of diagnosing an organism having a parasitic infection. The method involves contacting a sample from the organism with a *mlt* nucleic acid probe and detecting an increased level of a *mlt* nucleic acid in the sample relative to the level in a control sample not having a parasitic infection, thereby diagnosing the organism as having a parasitic infection.

In another aspect, the invention provides a method for diagnosing an organism having a parasitic infection. The method involves detecting an increased level of a MLT polypeptide in a sample from the organism, relative to the level in a control sample not having a parasitic infection, thereby diagnosing the organism as having a parasite infection. In one embodiment, this method of detection is an immunological method involving an antibody against a MLT polypeptide.

In other related aspects, the invention provides a biocide including a biocide excipient and a *mlt* nucleic acid, or portion thereof, that disrupts Ecdysozoan molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other related aspects, the invention provides a biocide including a biocide excipient and an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), or portion thereof, that disrupts Ecdysozoan molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other related aspects, the invention provides a biocide including a biocide excipient and a MLT polypeptide, or portion thereof, or an ortholog of a MLT polypeptide that disrupts Ecdysozoan molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other aspects, the invention provides an insecticide including an insecticide excipient and a MLT polypeptide or portion thereof, encoded by a MLT nucleic acid, or ortholog, that disrupts insect molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other related aspects, the invention provides an insecticide including an insecticide excipient and a *mlt* nucleic acid, or portion thereof, or ortholog, and disrupts insect molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other related aspects, the invention provides an insecticide including an insecticide excipient and an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA) that disrupts insect molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other aspects, the invention provides a nematicide including a nematicide excipient and an MLT polypeptide, or portion thereof, encoded by a *mlt* nucleic acid molecule, or ortholog.

In other related aspects, the invention provides a nematicide including a nematicide excipient and a *mlt* nucleic acid, or portion thereof, or ortholog, that disrupts nematode molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In other related aspects, the invention provides a nematicide including a nematicide excipient and an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), that disrupts nematode molting by at least 10%, 20%, 30%, 40%, 50%, 60%, or even 70%, 80%, 90%, 95%, or 99%.

In another related aspect, the invention provides a transgenic organism (e.g., Ecdysozoan) expressing a *mlt* nucleic acid molecule or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA) at a level sufficient to disrupt molting in the progeny of an Ecdysozoan (e.g., a nematode, a parasitic nematode, or an insect) breeding with the transgenic organism relative to a control nematode, parasitic nematode, or insect not bred with the organism. In various embodiments, the *mlt* nucleic acid molecule or RNA *mlt* nucleic acid inhibitor is expressed under the control of a conditional promoter. In some applications, for the control of a population of Ecdysozoan pests, a transgenic organism expressing a *mlt* nucleic acid molecule or an RNA

mlt nucleic acid inhibitor, or portion thereof, under the control of a conditional promoter, for example, may be released into an area infested with an Ecdysozoan pest (e.g., a nematode or insect pest). The transgenic organism transmits the *mlt* nucleic acid transgene during mating with wild-type Ecdysozoan pests to disrupt molting in the progeny, and controls a population of Ecdysozoan pests.

In other related aspects, the invention provides a transgenic plant expressing a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), or portion thereof, where a cell of the plant expresses the *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor at a level sufficient to disrupt molting in an Ecdysozoan (e.g., a nematode, a parasitic nematode, or an insect) that contacts (e.g., feeds on) the plant relative to a control nematode, parasitic nematode, or insect not contacted with the plant.

In other aspects, the invention provides a transgenic organism (e.g., insect or domestic mammal, such as a cow, sheep, goat, pig, or horse) expressing a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA), or portion thereof, at a level sufficient to disrupt molting in a nematode, a parasitic nematode, or an insect that contacts, (e.g., parasitizes or feeds on) the transgenic organism relative to a control nematode, parasitic nematode, or insect not contacted with the organism. Such transgenic organisms would be expected to be more resistant to parasitic nematode infection than control organisms not expressing a transgene. In preferred embodiments, the transgenic organism is an insect host organism (e.g., blackfly) capable of being infected with an Ecdysozoan parasite (e.g., nematode) that spends part of its life cycle as an insect parasite and part of its life cycle as a human parasite. Expression of the transgene in the transgenic host organism inhibits molting in the Ecdysozoan parasite, and is useful in controlling a human parasitic infection.

In preferred embodiments of the above aspects, a *mlt* nucleic acid is any one or all of the following B0024.14, C01H6.5, C09G5.6, C11H1.3, C17G1.6, C23F12.1, B0272.5, C34G6.6, C37C3.3, C42D8.5, C45B2.7, CD4.4, CD4.6, D1054.15, F08C6.1, F09B12.1, F11C1.6, F16H9.2, F18A1.3, F18C12.2, F20G4.1, F25B4.6, F29D11.1, F33A8.1, F33C8.3, F38H4.9, F40G9.1, F41C3.4, F41H10.7, F45G2.5, F49C12.12, F52B11.3, F53B8.1, F53G12.3, F54A5.1, F54C9.2, F56C11.1, F57B9.2, H04M03.4, H19M22.1, K04F10.4, K05C4.1, K06B4.5, K07C5.6, K07D8.1, K08B4.1, K09H9.6, M03F4.7, M03F8.3, M162.6, M6.1, M88.6, R05D11.3, R07E4.6, R11G11.1, T01C3.1, T01H3.1, T05C12.10, T14F9.1, T19B10.2, T23F2.1, T24H7.2, T27F2.1, W01F3.3, W08F4.6, W09B6.1, W10G6.3, Y111B2A.14, Y37D8A.10, Y38F2AL.3, Y48B6A.3, ZC101.2, ZK1073.1, ZK1151.1, ZK262.8, ZK270.1, ZK430.8, ZK686.3, ZK783.1, ZK970.4, C09F12.1, C09H10.2, C17H12.14, C37C3.2, D2085.1, EEED8.5, F10E9.7, F19F10.9, F28F8.5, F32D1.2, F35H10.4, F41E7.1, F42A8.1, F54B3.3, F55A3.3, F56F3.5, H06I04.4a, K06A4.6, K10D6.1, R06A10.1, T07D10.1, Y17G7A.2, Y23H5A.7, Y38F2AL.3, Y41D4B.21, Y41D4B.5, Y41D4B.5, Y45F10B.5, Y55H10A.1, ZK1236.3, ZK265.5, ZK265.6, ZK652.1, ZK1014.1,H15N14.1, F26H9.6, Y63D3A.5, C56C10.3,ZK180.4,F57H12.1, C39F7.4, Y113G7A.3, R160.1, C02C6.1, E03H4.8, F59E10.3, K12H4.4, D1014.3, C13B9.3, F43D9.3, B0336.10, B0393.1, C04F12.4, C23G10.3, D1007.6, F28D1.7, F35H10.4, F37C12.11, F37C12.9, F40F11.1, F53A3.3, T01C3.6, T05F1.3, Y45F10D.12, or Y54E10BR.5, B0513.1, R06A4.9, Y105E8B.1, Y47D3B.1, Y54F10AL.2, T17H7.3, H27M09.5, F45E10.2, F25H8.6, K04A8.6, ZC13.3, T19A5.3, F32D8.6, F53F4.3, F56C9.12, T25B9.10, ZK154.3, Y37D8A.19, Y37D8A.21, Y71F9AL.7, Y51H1A.3, W03F9.10, ZK945.2, ZK637.4, C30F8.2, F32H2.9, Y87G2A.5, Y53F4B.22, Y77E11A.13, C15H11.7, Y113G7B.23, C53H9.1, W09C5.6, T24B8.1, Y71A12B.1, C26C6.3, C42D8.5, F53G12.3, Y41D4B.10, F10C1.5, or a portion thereof, or an ortholog of any or all of these nucleic acids. In other embodiments, the *mlt* nucleic acid is a component of a secretory

pathway (e.g. ZK1014.1,H15N14.1, F26H9.6, Y63D3A.5, C56C10.3, ZK180.4, F57H12.1, C39F7.4, Y113G7A.3, R160.1, C02C6.1, E03H4.8, F59E10.3, K12H4.4, D1014.3, C13B9.3, and F43D9.3). In other embodiments, the *mlt* nucleic acid is a protein that functions in protein synthesis and produces *mlt* defects in less than 5% of larvae (e.g. B0336.10, B0393.1, C04F12.4, C23G10.3, D1007.6, F28D1.7, F35H10.4, F37C12.11, F37C12.9, F40F11.1, F53A3.3, T01C3.6, T05F1.3, Y45F10D.12).

In preferred embodiments of any of the above aspects, a *mlt* ortholog is any or all of the following *mlt* nucleic acids: M90806, NM_134578, AY075331, BG310588, BE758466, BG227161, BM346811, BG226227, BF169279, BE580288, BG893621, BQ625515, BI746672, AA471404, BE579677, BI500192, BI782938, BI073876, BF060055, AI723670, BI746256, BM882137, BM277122, BM880769, BI501765, BE581131, AI539970, BE580231, BE238916, AY060635, NM_143476, AC008339, L02793, NM_079167, J02727, NM_139674, NM_079763, NM_057268, NM_137449, NM_079419, NM_080092, AAF51201, NM_057698, NM_080132, NM_132335, AJ487018, NM_080072, AY094832, NM_057520, NM_136653, NM_078644, AY075331, M90806, NM_079419, NM_080092, AAF51201, NM_057698, NM_134578, AY071265, AY060235, NM_078577, NM_057621, AY089504, NM_135238, X78577, AY118647, NM_140652, AY113364, NM_079972, X58374, NM_132550, AY052122, AY060893, AY058709, AA161577, CAAC01000031, CAAC01000016, BI744615, BG224680, AW114337, BM281377, BU585500, BG577863, BQ091075, AW257707, BF014893, BQ613344, CAAC01000088, BG735742, CAAC01000028, AA110597, BI863834, AI987143, BI782814, BI744849, BG735807.

In other preferred embodiments of any of the above aspects, a Drosophila ortholog includes any or all of the following *mlt* nucleic acids: ref|NM_079167, gb|M90806, ref|NM_079419, ref|NM_080092, gb| AY075331, ref| NM_057698, ref|NM_132335, ref| NM_134871,

gb|AAF51201, ref|NM_136653, ref|NM_057520, ref| NM_080132, gb|AY094832, emb| AJ487018, ref|NM_080072, emb| AJ011925, ref|NM_078644, ref|NM_132550, ref|NM_079972, gb|AY089504, emb|X78577, gb|AY118647, gb| AY071265, ref|NM_140652, ref|NM_078577, emb|X58374, ref|NM_134578, gb|AY058709, gb|AY060235, gb|AY052122, AY060893, gb|AY113364, ref|NM_135238, ref|NM_057621, ref|NM_136498, ref| NM_143476, ref|NM_137449, gb|M16152, ref|NM_057268, ref|NM_139674, gb|L02793, gb|AY060635, gb|AC008339.

In other preferred embodiments of any of the previous aspect, the nucleic acid sequence is selected from those listed in Tables 1A, 1B, 4A-4D, or 7.

By "biocide" is meant any agent, compound, or molecule that slows, delays, inhibits, or arrests the growth, viability, molting, or reproduction of any Ecdysozoan by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, or even by as much as 70%, 80%, 90%, 95%, or 99%.

By "Ecdysozoan" is meant the clade of organisms that molt. Ecdysozoans include arthropods, tardigrades, onychophorans, nematodes, nematomorphs, kinorhynchs, loriciferans, and priapulids.

By "molting" is meant the shedding and synthesis of cuticle that occurs during the life cycle of an Ecdysozoan, such as a nematode or insect.

By "disrupts molting" is meant that the process of cuticle shedding is delayed, inhibited, slowed, or arrested. In some applications, the molting process is disrupted by larval arrest.

By "*mlt* nucleic acid" is meant a nucleic acid molecule, or an ortholog thereof, whose inactivation (e.g., by RNAi) results in a molting defect or larval arrest phenotype in an Ecdysozoan. RNAi of a *mlt* gene results in a Mlt phenotype or larval arrest phenotype in at least 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, or even in 70%, 80%, 90%, 95%, or 99% of the larvae exposed to dsRNA-expressing bacteria.

By "RNA *mlt* nucleic acid inhibitor" is meant a double-stranded RNA, antisense RNA, or siRNA, or portion thereof, that when administered to an Ecdysozoan results in a molting defect or larval arrest phenotype. Typically, an RNA *mlt* nucleic acid inhibitor comprises at least a portion of a *mlt* nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a *mlt* nucleic acid molecule. For example, a *mlt* nucleic acid molecule includes any or all of the nucleic acids listed in Tables 1A, 1B, 4A-4D, and 7.

By "MLT polypeptide" is meant any amino acid molecule encoded by a *mlt* nucleic acid. Typically, a MLT polypeptide functions in molting in an Ecdysozoan (e.g., nematode or insect).

By "parasite" is meant any multicellular organism that lives on or within the cells, tissues, or organs of a genetically distinct host organism.

By "parasitic nematode" is meant any nematode that lives on or within the cells, tissues, or organs of a genetically distinct host organism (e.g., plant or animal). For example, parasitic nematodes include, but are not limited to, any ascarid, filarid, or rhabditid (e.g., *Onchocerca volvulus, Ancylostoma, Ascaris, Ascaris lumbricoides, Ascaris suum, Baylisascaris, Baylisascaris procyonis, Brugia malayi, Dirofilaria, Dirofilaria immitis, Dracunculus, Haemonchus contortus, Heterorhabditis bacteriophora, Loa loa,* root-knot nematodes, such as *Meloidogyne, M. arenaria, , M. chitwoodi, M. graminocola, M. graminis, M. hapla, M. incognita, Necator, M. microtyla,* and *M. naasi,* cyst nematodes (for example, *Heterodera sp.* such as *H. schachtii, H. glycines, H. sacchari, H. oryzae, H. avenae, H. cajani, H. elachista, H. goettingiana, H. graminis, H. mediterranea, H. mothi, H. sorghi,* and *H. zeae,* or, for example, *Globodera sp.* such as *G. rostochiensis* and *G. pallida*) root-attacking nematodes (for example, *Rotylenchulus reniformis, Tylenchuylus semipenetrans, Pratylenchus brachyurus, Radopholus citrophilus, Radopholus similis, Xiphinema americanum, Xiphinema rivesi, Paratrichodorus minor, Heterorhabditis heliothidis,* and *Bursaphelenchus xylophilus*), and above-ground nematodes

(for example, *Anguina funesta*, *Anguina tritici*, *Ditylenchus dipsaci*, *Ditylenchus myceliphagus*, and *Aphenlenchoides besseyi*), *Parastrongyloides trichosuri*, *Pristionchus pacificus*, *Steinernema*, *Strongyloides stercoralis*, *Strongyloides ratti*, *Toxocara canis*, *Trichinella spiralis*, *Trichuris muris* or *Wuchereria bancrofti*).

By "nematicide" is meant any agent, compound, or molecule that slows, delays, inhibits, or arrests the growth, viability, molting, or reproduction of any nematode by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, or even by as much as 70%, 80%, 90%, 95%, or 99%.

By "insecticide" is meant any agent, compound, or molecule that slows, delays, inhibits, or arrests the growth, viability, molting, or reproduction of any insect by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, or even by as much as 70%, 80%, 90%, 95%, or 99%.

By "anti-parasitic" is meant any agent, compound, or molecule that ameliorates a parasitic infection in a host organism. In some applications, an anti-parasitic agent slows, delays, inhibits, or arrests the growth, viability, molting, or reproduction of a parasite in a host organism.

By "ortholog" is meant any polypeptide or nucleic acid molecule of an organism that is highly related to a reference protein or nucleic acid sequence from another organism. The degree of relatedness may be expressed as the probability that a reference protein would identify a sequence, for example, in a blast search. The probability that a reference sequence would identify a random sequence as an ortholog is extremely low, less than e^{-10}, e^{-20}, e^{-30}, e^{-40}, e^{-50}, e^{-75}, e^{-100}. The skilled artisan understands that an ortholog is likely to be functionally related to the reference protein or nucleic acid sequence. In other words, the ortholog and its reference molecule would be expected to fulfill similar, if not equivalent, functional roles in their respective organisms.

Drosophila melanogaster orthologs of *C. elegans mlt* genes include, but are not limited to, ref|NM_079167, gb|M90806, ref|NM_079419, ref|NM_080092, gb| AY075331, ref| NM_057698, ref|NM_132335, ref|

NM_134871, gb|AAF51201, ref|NM_136653, ref|NM_057520, ref| NM_080132, gb|AY094832, emb| AJ487018, ref|NM_080072, emb| AJ011925, ref|NM_078644, ref|NM_132550, ref|NM_079972, gb|AY089504, emb|X78577, gb|AY118647, gb| AY071265, ref|NM_140652, ref|NM_078577, emb|X58374, ref|NM_134578, gb|AY058709, gb|AY060235, gb|AY052122, AY060893, gb|AY113364, ref|NM_135238, ref|NM_057621, ref|NM_136498, ref| NM_143476, ref|NM_137449, gb|M16152, ref|NM_057268, ref|NM_139674, gb|L02793, gb|AY060635, and gb|AC008339.

Nematode orthologs of *C. elegans mlt* genes include, but are not limited to, BG310588 in *Onchocerca volvulus* (e^{-121}); BE758466 in *Brugia malayi* (e^{-104}); BG2271612 in *Strongyloides stercoralis* (e^{-84}); BM346811 in *Parastrongyloides trichosuri* (e^{-89}); BG226227 in *Strongyloides stercoralis* ($9e^{-24}$); BF169279 in *Trichuris muris* (4^{e-11}); BG893621 in *Strongyloides ratti* ($2e^{-20}$); BQ625515 in *Meloidogyne incognita* ($3e^{-25}$); BI746672 in *Meloidogyne arenaria* ($6e^{-31}$); AA471404 in *Brugia malayi* ($2e^{-68}$); BE579677 in *Strongyloides stercoralis* ($2e^{-53}$); BI500192 in *Pristionchus pacificus* ($2e^{-69}$); BI782938 in *Ascaris suum* ($9e^{-52}$); BI073876 in *Strongyloides ratti* ($1e^{-41}$); BF060055 in *Haemonchus contortus* ($4e^{-18}$); AI723670 in *Brugia malayi* ($8e^{-40}$); BI746256 in *Meloidogyne arenaria* ($3.^{00}e^{-15}$); BM882137 in *Parastrongyloides trichosuri* ($6e^{-33}$);BM277122 in *Trichuris muris* ($6e^{-15}$); BM880769 in *Meloidogyne incognita* ($3e^{-41}$); BI501765 in *Meloidogyne arenaria;* BE581131 in *Strongyloides stercoralis* ($1e^{-34}$); AI5399702 in *Onchocerca volvulus* (e^{-38}); BE5802318 in *Strongyloides stercoralis* (e^{-35}); BE2389166 *in Meloidogyne incognita* (e^{-17}); BE580288 in *Strongyloides stercoralis*, AA161577 in *Brugia malayi* (e^{-39}); CAAC01000016 in *C. briggsae*; BI744615 in *Meloidogyne javanica* (4e-44); BG224680 *Strongyloides stercoralis* ($4e^{-44}$); AW114337 *Pristionchus pacificus* (e^{-41}), BM281377 in *Ascaris suum* ($2e^{-41}$); BU585500 in *Ascaris lumbricoides*, BG577863 in *Trichuris muris* (e^{-24}); BQ091075 in *Strongyloides ratti* ($6e^{-14}$); AW257707 in *Onchocerca volvulus*; BF014893 in *Strongyloides stercoralis*

(7e-^{35}); BQ613344 in *Meloidogyne incognita* (5e^{-47}); CAAC01000088 in *C. Briggsae*, BG735742 in *Meloidogyne javanica* (4e^{-14}); CAAC01000028; AA110597 in *Brugia malayi* (3e^{-56}); BI863834 in *Parastrongyloides trichosuri* (3e^{-69}); AI987143 in *Pristionchus pacificus* (3e^{-60}); BI782814 in *Ascaris suum*; BI744849 in *Meloidogyne javanica*; and BG735807 in *Meloidogyne javanica* (6e^{-38}).

Of particular interest are orthologs of the following genes: B0024.14, C01H6.5, C09G5.6, C11H1.3, C17G1.6, C23F12.1, B0272.5, C34G6.6, C37C3.3, C42D8.5, C45B2.7, CD4.4, CD4.6, D1054.15, F08C6.1, F09B12.1, F11C1.6, F16H9.2, F18A1.3, F18C12.2, F20G4.1, F25B4.6, F29D11.1, F33A8.1, F33C8.3, F38H4.9, F40G9.1, F41C3.4, F41H10.7, F45G2.5, F49C12.12, F52B11.3, F53B8.1, F53G12.3, F54A5.1, F54C9.2, F56C11.1, F57B9.2, H04M03.4, H19M22.1, K04F10.4, K05C4.1, K06B4.5, K07C5.6, K07D8.1, K08B4.1, K09H9.6, M03F4.7, M03F8.3, M162.6, M6.1, M88.6, R05D11.3, R07E4.6, R11G11.1, T01C3.1, T01H3.1, T05C12.10, T14F9.1, T19B10.2, T23F2.1, T24H7.2, T27F2.1, W01F3.3, W08F4.6, W09B6.1, W10G6.3, Y111B2A.14, Y37D8A.10, Y38F2AL.3, Y48B6A.3, ZC101.2, ZK1073.1, ZK1151.1, ZK262.8, ZK270.1, ZK430.8, ZK686.3, ZK783.1, ZK970.4, C09F12.1, C09H10.2, C17H12.14, C37C3.2, D2085.1, EEED8.5, F10E9.7, F19F10.9, F28F8.5, F32D1.2, F35H10.4, F41E7.1, F42A8.1, F54B3.3, F55A3.3, F56F3.5, H06I04.4a, K06A4.6, K10D6.1, R06A10.1, T07D10.1, Y17G7A.2, Y23H5A.7, Y38F2AL.3, Y41D4B.21, Y41D4B.5, Y45F10B.5, Y55H10A.1, ZK1236.3, ZK265.5, ZK265.6, ZK652.1, Y54E10BR.5, B0513.1, R06A4.9, Y105E8B.1, Y47D3B.1, Y54F10AL.2, T17H7.3, H27M09.5, F45E10.2, F25H8.6, K04A8.6, ZC13.3, T19A5.3, F32D8.6, F53F4.3, F56C9.12, T25B9.10, ZK154.3, Y37D8A.19, Y37D8A.21, Y71F9AL.7, Y51H1A.3, W03F9.10, ZK945.2, ZK637.4, C30F8.2, F32H2.9, Y87G2A.5, Y53F4B.22, Y77E11A.13, C15H11.7, Y113G7B.23, C53H9.1,

W09C5.6, T24B8.1, Y71A12B.1, C26C6.3, C42D8.5, F53G12.3, Y41D4B.10, and F10C1.5. Other *mlt* genes may be identified using the methods of the invention described herein.

By "portion" is meant a fragment of a protein or nucleic acid that is substantially identical to a reference protein or nucleic acid, and retains at least 50% or 75%, more preferably 80%, 90%, or 95%, or even 99% of the biological activity of the reference protein or nucleic acid using a molting assay as described herein.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes, which, in the naturally occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation).

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant

nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80%, and most preferably 90% or even 95% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a polynucleotide molecule encoding (as used herein) a polypeptide of the invention.

By "positioned for expression" is meant that the polynucleotide of the invention (e.g., a DNA molecule) is positioned adjacent to a DNA sequence that directs transcription and translation of the sequence (i.e., facilitates the production of, for example, a recombinant polypeptide of the invention, or an RNA molecule).

By "specifically binds" is meant a compound or antibody which recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

By "derived from" is meant isolated from or having the sequence of a naturally occurring sequence (e.g., a cDNA, genomic DNA, synthetic, or combination thereof).

By "immunological assay" is meant an assay that relies on an immunological reaction, for example, antibody binding to an antigen. Examples of immunological assays include ELISAs, Western blots, immunoprecipitations, and other assays known to the skilled artisan.

By "anti-sense" is meant a nucleic acid sequence, regardless of length, that is complementary to the coding strand or mRNA of a nucleic acid sequence. In one embodiment, an antisense RNA is introduced to an individual cell, tissue, organ, or to a whole animals. Desirably the anti-sense nucleic acid is capable of decreasing the expression or biological activity of a nucleic acid or amino acid sequence. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The anti-sense nucleic acid may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By "double stranded RNA" is meant a complementary pair of sense and antisense RNAs regardless of length. In one embodiment, these dsRNAs are introduced to an individual cell, tissue, organ, or to a whole animals. For

example, they may be introduced systemically via the bloodstream. Desirably, the double stranded RNA is capable of decreasing the expression or biological activity of a nucleic acid or amino acid sequence. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The anti-sense nucleic acid may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By "siRNA" is meant a double stranded RNA that complements a region of an mRNA. Optimally, an siRNA is 21, 22, 23, or 24 nucleotides in length and has a 2 base overhang at its 3' end. siRNAs can be introduced to an individual cell, tissue, organ, or to a whole animals. For example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity. Desirably, the siRNA is capable of decreasing the expression or biological activity of a nucleic acid or amino acid sequence. In one embodiment, the decrease in expression or biological activity is at least 10%, relative to a control, more desirably 25%, and most desirably 50%, 60%, 70%, 80%, 90%, or more. The siRNA may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the

absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur

at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci.*, USA 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell and typically becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene that is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. A transgene of the invention may encode a MLT polypeptide or an RNA *mlt* nucleic acid inhibitor.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell, or part of a heritable extra chromosomal array. As used herein, transgenic organisms may be either transgenic vertebrates, such as domestic mammals (e.g., sheep, cow, goat, or horse), mice, or rats, transgenic invertebrates, such as insects or nematodes, or transgenic plants.

By "cell" is meant a single-cellular organism, cell from a multi-cellular organism, or it may be a cell contained in a multi-cellular organism.

By "differentially expressed" is meant a difference in the expression level of a nucleic acid. This difference may be either an increase or a decrease in expression, when compared to control conditions.

By "therapeutic compound" is meant a substance that affects the function of an organism. Such a compound may be, for example, an isolated naturally occurring, semi-synthetic, or synthetic agent. For example, a therapeutic compound may be a drug that targets a parasite infecting a host organism. A therapeutic compound may decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of disease, disorder, or infection in a eukaryotic host organism.

The invention provides for compositions and methods useful for inhibiting molting in an Ecdysozoan (e.g., a parasitic nematode, nematode or insect). Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Brief Description of the Drawings

FIGURES 1A-1E are micrographs showing Mlt phenotypes associated with RNAi of *mlt-24, mlt-18, mlt-12,* and *mlt-13* in nematodes visualized using Nomarski optics. Figures 1A and 1B are micrographs showing the Mlt phenotype of a *mlt-24(RNAi)* nematode. Figure 1C is a micrograph showing the Mlt phenotype of a *mlt-18(RNAi)* nematode. Figure 1D is a micrograph showing the Mlt phenotype of a *mlt-12(RNAi)* nematode. Figure 1E is a micrograph showing the Mlt phenotype of a *mlt-13(RNAi)* nematode. Black arrows indicate where excess cuticle remains attached to the larvae.

FIGURES 2A-2D show that molting genes are expressed in a pulse before each molt. Figure 2A is a series of micrographs showing fluorescence from *mlt-12::gfp-pest* early in L1, at the L1/L2 molt, and early in L2. The L2 larvae was fluorescent before molting. Black arrows indicate cuticle separated from the body. Figures 2B and 2C are graphs showing the percentage of worms that were fluorescent over time, on a scale normalized to the period between molts for each worm under observation. The bar at the top of the graph indicates the worm's developmental stage. Figure 2B shows results for *Ex*[*mlt-12::gfp-pest*] (dashed line) or *Ex*[*mlt-10::gfp-pest*] (solid line) larvae

scored for detectable fluorescence and for molting once per hour from late in the L1 stage until early adulthood. Figure 2C shows cycling fluorescence in worms expressing *mlt- 13::gfp-pest* (dashed line) or *mlt-18::gfp-pest* (solid line), observed in the hypodermis and seam cells. Figure 2C shows Northern analysis of *mlt-10* messenger RNA levels. Ribosomal RNA stained with ethidium-bromide provides a loading control.

FIGURES 3A-3H are micrographs showing GFP fluorescence associated with *Pmlt-18::GFP-PEST* and *Pmlt-13::GFP-PEST* expression in transgenic nematodes. Figures 3A, 3C, and 3E are micrographs showing GFP fluorescence in transgenic *Pmlt-18::GFP-PEST* expressing nematodes during early L1, L1/L2 molt, and early L2. Figures 3B, 3D, and 3F are micrographs of nematodes visualized using Nomarski optics. The black arrow in Figure 2D indicates shedding of the cuticle at the L1/L2 molt. Worms were synchronized after hatching and monitored through larval development. Figures 3G and 3H are micrographs of nematodes showing GFP fluorescence in transgenic *Pmlt-13::GFP-PEST* expressing nematodes during early L2 and L1/L2 molt. The inset in Figures 3G and 3H is a micrograph of the transgenic nematode visualized using Nomarski optics.

FIGURE 4A is a graph showing the percentage of animals that were fluorescent before a defective molt, normalized to the percentage of control larvae that were fluorescent before molting from the same stage. *Ex*[*mlt-12::gfp-pest*], indicated with black bars, or *Ex*[*mlt-10::gfp-pest*] larvae, indicated with gray bars, were fed bacteria expressing dsRNA for each gene indicated. "n" indicates the number of larvae observed. Pairwise chi-square tests indicated that the decreased fraction of fluorescent *Ex*[*mlt-12::gfp-pest*] larvae after RNAi of *nhr-23* or *acn-1*, and of fluorescent *Ex*[*mlt-10::gfp-pest*] larvae after RNAi of *nhr-23*, *acn-1*, or *mlt-12*, relative to control animals, is significant, with $p<.001$ in all 5 tests.

FIGURE 4B is a graph that shows the percentage of late L4 larvae with detectable fluorescence, for selected gene inactivations. *Ex*[*mlt-10::gfp-pest*] larvae were fed bacteria expressing dsRNA for each gene indicated. Values represent the weighted average of two independent trials.

FIGURES 5A-5G are a series of micrographs showing expression of molting gene *gfp* fusion genes in worms. Figures 5A-C show expression from *mlt-24::gfp-pest.* Figure 5A shows fluorescence in the hypodermis (arrow) and seam cells (arrowhead) of an L4 larvae. Figure 5B shows fluorescence in the rectal gland. The solid line traces the tail of the worm, the dashed line outlines the intestine. Figure 5C is a pair of micrographs showing fluorescence and Nomarski images of the vulva of a young adult. Figure 5D-5F are micrographs showing expression of *acn-1::gfp-pest* in a worm. Figure 5D shows fluorescence in the excretory gland, duct, and pore cells (Exc), and in the glial cells (G) of interlabial neurons of larvae (lateral view). Figure 5E shows fluorescence in the excretory gland (GN) and duct cells. A solid line traces the worm, and a dashed line outlines the posterior bulb of the pharynx. Figure 5F shows fluorescence in the hypodermis and seam cells of a late L1 larvae. Figure 5G shows fluorescence from *mlt-18::gfp-pest* in the hypodermis (arrow) and seam cells (arrowhead) of a late L1 larvae. Figure 5H shows fluorescence from *mlt-13::gfp* in the hypodermis and seam cells of a late L3 larvae. The seam cell fluorescence from *mlt-24::gfp-pest* was observed only near the L4/Adult molt, when the cells terminally differentiate and fuse, whereas seam-cell fluorescence from *mlt-13::gfp-pest* and *mlt-18::gfp-pest* was observed most often near larval-to-larval molts, when the cells divide. The anterior of the worm is at the right in all panels.

Description of the Invention

The post-embryonic development of *C. elegans* proceeds through four larval stages that are separated by periodic molts when the collagen-like cuticle that encases the worm's body is shed and synthesized anew. As reported in more detail below, genes important for molting in *C. elegans* were identified by the present inventors through a genome-wide screen using bacterial-mediated RNA-interference (RNAi) to reduce gene function. Molting (*mlt)* gene inactivation by RNAi caused larvae to become trapped in old cuticle while attempting to molt. Inactivation of these genes, their orthologs in Ecdysozoans, or their encoded proteins by genetic or chemical means is expected to block molting and larval development in virtually any Ecdysozoan (e.g., nematodes and insects).

Four classes of genes central to molting function have been identified. The first class includes *mlt* genes that function specifically in nematodes (e.g., C09G5.6, C17G1.6, C23F12.1, C34G6.6, F08C6.1, F09B12.1, F16B4.3, F18A1.3, F45G2.5, F49C12.2, F53B8.1, H04M03.4, H19M22.2, K07D8.1, M6.1, M88.6, T05C12.10, W01F3.3, W08F4.6, Y111B2A.14, ZK262.8, ZK270.1, and ZK430.8). The protein products of such genes are likely to function in the execution phase of nematode molting and represent attractive targets for the development of highly specific nematicides. The second class includes *mlt* genes conserved in insects and nematodes, but not present in humans or yeast (e.g., C01H6.5, F11C1.6, F52B11.3, and ZK686.3). Nematicides and insecticides targeting such *mlt* genes, or their orthologs in insects or parasitic nematodes, are likely to specifically disrupt molting processes common to Ecdysozoans, and given this specificity are unlikely to adversely effect human health. The third class includes *mlt* genes whose inactivation by RNA results in highly penetrant molt defects (e.g., those molt genes listed in Tables 1A and Table 1B). Tables 1A and 1B include genes not previously identified as being involved in molting (e.g., B0024.14, C09G5.6, C11H1.3, C23F12.1, B0272.5, C34G6.6, C37C3.3, C42D8.5, CD4.4, CD4.6,

D1054.15, F08C6.1, F09B12.1, F16H9.2, F18A1.3, F20G4.1, F25B4.6, F33A8.1, F33C8.3, F38H4.9, F40G9.1, F41C3.4, F41H10.7, F45G2.5, F49C12.12, F52B11.3, F53B8.1, F54A5.1, F54C9.2, F57B9.2, H04M03.4, H19M22.1, K05C4.1, K06B4.5, K07C5.6, K07D8.1, K08B4.1, K09H9.6, M03F4.7, M03F8.3, M162.6, M6.1, M88.6, R05D11.3, R07E4.6, R11G11.1, T01C3.1, T01H3.1, T14F9.1, T19B10.2, T23F2.1, T24H7.2, W01F3.3, W08F4.6, W09B6.1, W10G6.3, Y111B2A.14, Y37D8A.10, Y38F2AL.3, Y48B6A.3, ZC101.2, ZK1073.1, ZK1151.1, ZK262.8, ZK430.8, ZK686.3, ZK783.1, ZK970.4, Y54E10BR.5, B0513.1, R06A4.9, Y105E8B.1, Y47D3B.1, Y54F10AL.2, T17H7.3, H27M09.5, F45E10.2, F25H8.6, K04A8.6, ZC13.3, T19A5.3, F32D8.6, F53F4.3, F56C9.12, T25B9.10, ZK154.3, Y37D8A.19, Y37D8A.21, Y71F9AL.7, Y51H1A.3, W03F9.10, ZK945.2, ZK637.4, C30F8.2, F32H2.9, Y87G2A.5, Y53F4B.22, Y77E11A.13, C15H11.7, Y113G7B.23, C53H9.1, W09C5.6, T24B8.1, Y71A12B.1, C26C6.3, C42D8.5, F53G12.3, Y41D4B.10, and F10C1.5) as well as genes not previously suggested as targets for insecticides or nematicides (e.g., C01H6.5, C17G1.6, C45B2.7, F11C1.6, F18C12.2, F29D11.1, F53G12.3, F56C11.1, K04F10.4, T05C12.10, T27F2.1, Y23H5A.7, and ZK270.1). A fourth class includes *mlt* genes involved in the neuroendocrine control of molting. Such genes are expected to be conserved between nematodes and insects (e.g., *Drosophila*). *C. elegans* neuronal control genes are often refractory to RNAi; thus, RNAi against neuroendocrine control genes is likely to effect molting in only a small percentage of larvae. Neuroendocrine control genes will likely be identified among *mlt* genes whose inactivation by RNA interference results in molting defects in less than 5% of larvae (e.g., C09F12.1, C09H10.2, C17H12.14, C37C3.2, D2085.1, EEED8.5, F10E9.7, F19F10.9, F28F8.5, F32D1.2, F35H10.4, F41E7.1, F42A8.1, F54B3.3, F55A3.3, F56F3.5, H06I04.4a, K06A4.6, K10D6.1, R06A10.1, T07D10.1, Y17G7A.2,

Y38F2AL.3, Y41D4B.21, Y41D4B.5, Y41D4B.5, Y45F10B.5, Y55H10A.1, ZK1236.3, ZK265.5, ZK265.6, ZK652.1, F32D8.6, F53F4.3, F56C9.12, T25B9.10, ZK154.3, Y37D8A.19, Y37D8A.21, Y71F9AL.7, Y51H1A.3, W03F9.10, ZK945.2, ZK637.4, C30F8.2, F32H2.9, Y87G2A.5, Y53F4B.22, Y77E11A.13, C15H11.7, Y113G7B.23, C53H9.1, W09C5.6, T24B8.1, and Y71A12B.1. Additional *mlt* genes may be identified using a nematode strain having enhanced susceptibility to RNAi.

These compositions and methods are described further below.

RNAi Library Screen

To systematically identify genes required for molting in *C. elegans*, a library of 16,757 bacterial clones was used. Each HT115(DE3) *E. coli* clone (Timmons et al., *Gene* 263:103-112, 2001) expressed a double-stranded RNA corresponding to a single open reading frame (ORF) predicted in the *C. elegans* genome (Fraser et al., *Nature*, 408:325-30, 2000). Approximately 85% of all ORFs predicted to be present in the genome of *C. elegans* were represented in this library. Approximately 2,000 additional clones, which are publicly available through the Vidal lab ORFeome project at Harvard University (Orfeome project, Harvard University website) were also screened. The genes listed in Table 1B were identified in this screen.

Briefly, the bacterial colonies from each plate of the library were inoculated into 96-well microtiter dishes containing 300 ul of LB with 50 ug/ml of ampicillin. The bacteria were then cultured for approximately sixteen hours at 30°C. 30ul of each overnight culture was plated onto a single well of a 24-well plate containing Nematode Growth Medium (NGM)-agar, IPTG (8 mM final concentration), and carbenicillin (25 ug/ml).

Early L1 larvae from wild-type (N2) worms were isolated using standard techniques, and approximately twenty larvae were added to each well. The worms were then incubated in individual wells at 20°C for two and a half days with one of the 16,757 bacterial clones serving as a food source.

Nematodes in each well were examined for molting defects by visual inspection using a standard light microscope. These assays were carried out "blind" (i.e., the researcher examining the nematode's molting phenotype was unaware of the identity of the bacterial clone present in the well at the time the phenotype was scored). A molting defect was identified by the presence of larvae with unshed cuticle attached to their bodies (the Mlt phenotype). Molting defects were never observed in control larvae fed on bacteria transformed with an empty vector. The majority of control larvae grew into gravid adult nematodes and sired progeny during the time of observation. As a positive internal control for the efficacy of post-embryonic RNAi, wild-type N2 larvae were concurrently fed HT115(DE3) bacteria expressing dsRNA corresponding to a known *mlt* gene, *lrp-1*.

C. elegans genes required for molting are listed in Tables 1A, 1B, 4A-4D, 7, and 8. Open reading frames initially identified as causing a Mlt phenotype were verified by re-screening two additional times. The identity of the gene represented by each bacterial colony was verified by sequencing. This was accomplished by sequencing the insert in the plasmid DNA isolated from the bacterial clone using primers complementary to flanking sequence present in the vector L440 (Timmons et al., Nature 391:806-811, 1998).

To evaluate the dauer molt, hatchlings of the temperature-sensitive, dauer constitutive mutants *daf-2(e1370)* and *daf-7(e1372)* were fed bacterial clones expressing dsRNA for each molting gene and cultivated at restrictive temperature (25°C) for 3 days, such that control animals all became dauers. Animals were then shifted to permissive temperature (15°C) for 2 days, allowing control animals to molt to the L4 stage. Observation of L2d or dauer larvae with the Mlt phenotype, in either genetic background, indicated that a given gene inactivation disrupted the L2d/dauer or dauer/L4molt.

Nomenclature

C. elegans genes whose inactivation by RNAi caused a molting defect, or Mlt phenotype, are shown in Tables 1A, 1B, 4A-4D, 7 and 8. These genes are identified by a *C. elegans* gene name and by an open reading frame number. Genes not previously assigned a *C. elegans* gene name are identified herein as *mlt-12* to *mlt-93*. Eleven genes identified in our screen had been previously identified as functioning in molting, but had not been previously identified as targets for a nematicide, insecticide, or other compound that inhibits molting. These genes include C01H6.5 (*nhr-23*), C45B2.7 (*ptr-4*), F11C1.6 (*nhr-25*), F18C12.2 (*rme-8*), F29D11.1 (*lrp-1*), F53G12.3, F56C11.1, K04F10.4 (*bli-4*), T05C12.10 (*qhg-1*), T27F2.1 (*C. elegans Skip*), and ZK270.1 (*ptr-23*). Orthologs of these genes were not previously identified. Some genes not previously identified as functioning in molting had been previously assigned a *C. elegans* gene name. In keeping with *C. elegans* nomenclature practices, genes previously assigned a *C. elegans* gene name have not been renamed.

Mlt Phenotypes

Post-embryonic RNAi against *mlt* genes listed in Tables 1A and 1B produced molting-specific defects in 5-100% of larvae (Table 1A and Table 1B). The majority of these worms also exhibited a larval arrest phenotype. This list identifies target genes by *C. elegans* cosmid name and open reading frame number. Homology searches using the blast algorithm and information available at wormbase (www.wormbase.org), a central repository of data on *C. elegans,* were used to identify the function of encoded proteins. At least three *mlt* genes, *mlt-24*, *mlt-25*, and *mlt-27*, encode proteins predicted to function as secreted proteases. These proteases are likely to function in the process of cuticle release, or, possibly, in the processing of peptide molting hormones.

Table 1A RNAi Produced Molt Defects in 5-100% of Exposed Larvae

Gene	ORF	Accession No.	Function	Reference for Mlt phenotypee
mlt-19	B0024.14	ref|NM_073255	Pro-collagen	
nhr-23	C01H6.5	ref|NM_059638	nuclear hormone receptor transcription factor	Kostrouchova et al., 1998[1]
bli-1	C09G5.6	ref|NM_063910	cuticle collagen	
	C11H1.3	ref|NM_077984		
mlt-24	C17G1.6	ref|NM_077268	Metalloprotease, secreted	Morita et al., 2002[2]
mlt-20	C23F12.1	ref|NM_077180	endothelial actin-binding protein repeats	
mlt-21	B0272.5	same as above	endothelial actin-binding protein repeats	
mlt-14	C34G6.6	ref|NM_059305	repetitive Cys motifs; 4 PAN domains	
mlt-22	C37C3.3			
mlt-27	C42D8.5	ref|NM_076466	Angiotension converting enzyme, metalloprotease	
ptr-4	C45B2.7	ref|NM_076612	sterol-sensing domain	Zugasti et al., 2002[3]
mlt-23	CD4.4	ref|NM_072073	coiled coil	
mlt-28	CD4.6	pir| T32525	protease	
mlt-29	D1054.15	ref|NM_073362	G-protein beta WD-40 repeats beta-transducin-like	
mlt-21	C26C6.3	NM_059708.	Astacin metalloprotease	
acn-1	C42D8.5	NM_076466	Angiotension converting enzyme	
mlt-20	F08C6.1	ref|NM_076885	ADAM/reprolysin metalloprotease, 12 of Thrombospondin type I domain	
mlt-13	F09B12.1	ref|NM_078111	MAM domains, secreted	
nhr-25	F11C1.6	ref| NM_077761	nuclear hormone recptor	Gissendanner and Sluder, 2000[4]
mlt-30	F16H9.2	ref| NM_077722	nuclear hormone receptor	
lir-1	F18A1.3	emb|AJ130959	Transcription factor like lin-26	
rme-8	F18C12.2	gb|AF372457	endocytosis DNAJ domain	Zhang et al., 2001[5]
mlt-31	F20G4.1	ref|NM_059784		
mlt-32	F25B4.6	ref|NM_072095	hydroxymethlglutaryl-CoA synthase	
lrp-1	F29D11.1	ref|NM_059726	LDL-receptor related	Yochem et al., 1999[6]
let-858	F33A8.1	ref|NM_063962		
mlt-33	F33C8.3	ref|NM_078044	tetraspanin	
mlt-34	F38H4.9	ref|NM_069846	Hs P2AB serine/threonine phosphatase	
mlt-35	F40G9.1	ref|NM_064775	Ankryin repeats	
mlt-36	F41C3.4	ref|NM_062446		
elo-5	F41H10.7	ref| NP_500793	GNS1/SUR4 family	
mlt-17	F45G2.5	ref|NM_067371	SS pancreatic trypsin inhibitor	
mlt-38	F49C12.12	ref|NM_069234	transmembrane protein	
mlt-15	F52B11.3	ref|NP_502699	4 PAN domains. Secretory protein	

mlt-39	F53B8.1	pir||T22551	Hu PLEC1 orthologue; plectin, kakapo homolog	
mlt-40	F53G12.3	ref|NM_058283	NADPH oxidase	Fraser, 2000[7]
mlt-41	F54A5.1	ref|NM_058402		
stc-1	F54C9.2	ref|NM_063407	Heat shock 70Kd protein (HSP70)	
	F53G12.3		animal haem peroxidase; gp91/phox1	
DuOx	F56C11.1	ref|NM_058285	NADPH oxidase; animal haem peroxidase; gp91/phox1	Fraser, 2000[7]
mlt-42	F57B9.2	ref|NM_066115	Tx human 1 Proline Rich, 1 Glycosylytransferase family 5	
mlt-43	H04M03.4	ref|NP_500884		
let-805	H19M22.1	ref|NM_065198	myotactin form A	
bli-4	K04F10.4	ref|NM_059427	subtilase protease	Thacker et al., 1995[8]
mlt-44	K05C4.1	pir|T23336	proteasome subunit	
mlt-45	K06B4.5	ref|NM_074499	nuclear hormone receptor	
mlt-46	K07C5.6	ref|NM_073260	zinc finger	
mup-4	K07D8.1	ref|NM_066244	mup-4 ion-channel SEA domains, Ca-binding EGF domains	
lag-1	K08B4.1	ref|NM_068515	DNA-binding protein, IPT/TIG domain	
mlt-47	K09H9.6	ref|NM_058707	homologueof Dm Peter Pan, which is required for larval growth	
mlt-48	M03F4.7	ref|NM_076443	calcium binding protein, EF-hand family 13x	
mlt-49	M03F8.3	ref|NM_072146	crn HAT (Half-A-TPR) repeat 10x, TPR repeat 3x	
mlt-50	M162.6	ref|NM_075434		
ifc-2	M6.1	ref|NM_075732	intermediate filament protein A	
pan-1	M88.6	ref|NM_065523	lecuine-rich repeats	
ran-4	R05D11.3	ref|NM_059921	Nuclear import; Nuclear Transport Factor 2 (NTF2) homologue	
kin-2	R07E4.6	ref|NM_076598		
mlt-52	R11G11.1	ref| NM_070836	nuclear homrone receptor	
mlt-53	T01C3.1	ref|NM_074284	WD domain, G-beta repeats x13	
mlt-54	T01H3.1	ref|NM_063258	proteolipid protein PPA1 like protein	
	Y41D4B.10	NM_067707	Delta-serrate ligand precursor	
qhg-1	T05C12.10	ref|NM_063324	hedgehog-like, hint module	Wang et al., 1999[9]
mlt-55	T14F9.1	ref|NM_076011	ATPase subunit	
mlt-56	T19B10.2	ref|NM_073447	secretory protein	
mlt-57	T23F2.1	ref|NM_076531	glycosyltransferase	
mlt-58	T24H7.2	ref| NM_062848	Heat shock protein hsp70, Cytochrome b/b6	
Ce Skip	T27F2.1	ref|NM_073549	Drosophila puff specific protein BX42 like	Kostrouchova et al., 2002[10]
	F10C1.5	NM_062737	DSX DNA binding domain	

mlt-18	W01F3.3	ref|NM_075592	multiple BPTI-like domains, secretory protein	
mlt-12	W08F4.6	ref|NM_061358	novel secretory protein	
mlt-59	W09B6.1	ref|NM_061521	acetyl-CoA carboxylase	
ifa-2	W10G6.3	ref|NM_078247	intermediate filament protein	
pqn-80	Y111B2A.14	ref|NM_067244	prion-like	
mlt-60	Y37D8A.10	ref|NM_067275	transmembrane protein	
mlt-61	Y38F2AL.3	ref| NM_067786	ATPase	
mlt-62	Y48B6A.3	ref|NM_067371	5'-3' exonuclease domain; eggshell protein	
unc-52	ZC101.2	ref|NM_064645	basement membrane proteoglycan	
mlt-63	ZK1073.1	ref|NM_078233		
mlt-64	ZK1151.1	ref|NM_060597	plectrin	
mlt-65	ZK262.8	ref| NM_075208	Myosin head (motor domain)	
ptr-23	ZK270.1	ref|NM_061202	sterol-sensing domain	Schulze et al., 2002[11]
mlt-11	ZK430.8	ref|NM_062376	animal haem peroxidase; ShTk domain	
mlt-67	ZK686.3	ref|NM_066290	Ankryin repeat	
mlt-16	ZK783.1	ref|NM_066269	ECM microfibril component (Hs FBN-1 homolog)	
mlt-68	ZK970.4	ref|NM_063816	H+-transporting ATPase	

[1]Kostrouchova et al., *Proc. Natl. Acad. Sci.* 99:9554-9559,2002
[2]Morita et al., EMBO 23:1063-1073.
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[7]Fraser et al., *Nature*, 408:325-30, 2000
[8]Thacker et al., *Genes Dev.* 9: 956-71, 1995
[9]Wang et al., 1999, International Worm Meeting
[10] Kostrouchova et al., *Proc. Natl. Acad. Sci.* 98:7360-5, 2001
[11]Schulze et al., 2002 European Worm Meeting

Table 1B: Genes identified in RNAi screen of clones from Vidal Orfeome Project

Predicted Gene	Gene name	Accession #	Brief Molecular I.D./ Domains
High frequency of Mlt phenotype			
Y54E10BR.5		refINM_058691	Signal Peptidase
B0513.1	*gei-5*	refINM_070273	GEX-3 interacting protein
R06A4.9		refINM_064584	WD domain, G beta repeats, HMGI/Y DNA binding domain
Y105E8B.1	*lev-11*	refINM_061138	tropomyosin
Y47D3B.1		refINM_067064	DUF23
Y54F10AL.2	*est-1*	refINM_065164	telomerase subunit
T17H7.3		refINM_064848	
H27M09.5		refINM_059558	novel
F45E10.2		refINM_063970	solute carrier family 22 member
F25H8.6		refINM_069384	DNA binding, BED zinc finger
K04A8.6		refINM_072260	F-box
ZC13.3		refINM_075772	MAM domain
T19A5.3		refINM_072907	novel
low frequency of Mlt phenotype			
F32D8.6	*emo-1*	refINM_073377	Protein translocation - Sec61 ortholog
F53F4.3		refINM_073966	novel
F56C9.12		refINR_001470	novel
T25B9.10		refINM_069598	endo/exonuclease phosphatase family
ZK154.3	*mec-7*	refINM_076912	beta-tubulin
Y37D8A.19		refINM_067286	novel secreted protein
Y37D8A.21		refINM_067285	RNA binding, RNP domain
Y71F9AL.7		refINM_058666	novel transmembrane protein
Y51H1A.3		refINM_064506	NADH dehydrogenase 1 beta subcomplex 8 19kDa like
W03F9.10		refINM_070740	DUF382, Proline rich, PSP, HMG-1 DNA binding
ZK945.2	*pas-7*	refINM_063776	proteosome alpha subunit
ZK637.4		refINM_066563	novel putative nuclear protein
C30F8.2		refINM_059114	H+ transporting ATPase C subunit
F32H2.9	*tba-6*	refINM_060018	tubulin alpha
Y87G2A.5	*vrs-2*	refINM_060976	cytoplasmic valyl tRNA syhtethase
Y53F4B.22	*arp-1*	refINM_064707	actin like
Y77E11A.13	*npp-20*	refINM_067686	nuclear core protein, related to essential transport protein SEC1
C15H11.7	*pas-1*	refINM_074170	26s proteosome subunit
Y113G7B.23	*psa-1*	refINM_075505	SWI/SNF complex chromatin remodeling
C53H9.1	*rpl-27*	refINM_058504	large ribosomal subunit 27
W09C5.6	*rpl-31*	refINM_060990	large ribosomal subunit 31
T24B8.1	*rpl-32*	refINM_063533	large ribosomal subunit 32
Y71A12B.1	*rps-6*	refINM_061034	small ribosomal subunit S6

Cuticle Retention Phenotypes

All Mlt larvae failed to fully shed their cuticles. For example, RNAi against *mlt-12, mlt-13*, *mlt-18*, and *mlt-24* resulted in larvae partially encased in a sheath of unshed cuticle (Figures 1A-1E). The Mlt phenotype observed in these animals resembled the phenotype of *lrp-1*(RNAi) nematodes. *lrp-1* was previously shown to be required for molting (Yochem et al., *Development*, 126: 597-606, 1999).

Interestingly, specific differences were observed in cuticle retention among Mlt larvae. The tissue of *mlt-13(RNAi)* animals remained tethered to old cuticle expelled from the buccal cavity, suggesting a defect early in the execution of molting (Figure 1E). In contrast, *unc-52(RNAi)* nematodes arrested with sheaths of cuticle extending from their posteriors, and appeared paralyzed except for small head movements. The phenotype of *unc-52(RNAi)* nematodes suggested a defect in the final stages of ecdysis. Undetached cuticle was observed around the most anterior portion of *mlt-12(RNAi)* animals (Figure 1D). This anterior region corresponds to the location of the cells hyp2 through hyp6. Approximately 20% of *mlt-24(RNAi)* animals had cuticular sheaths wrapped around their mid-sections (Figures 1A and 1B). The discovery of phenotypic classes among Mlt larvae indicated that sets of *mlt* genes likely act together at specific steps of ecdysis, or that some *mlt* genes are required for apolysis of cuticle covering only one or two regions of the body. Further, most, if not all, genes uncovered appear essential for all four molts, since their inactivation produces molting-defective larvae at several developmental stages. The majority of gene inactivations also disrupted molts into, or out of, dauer, an alternative developmental stage that is adapted for survival in unfavorable conditions and resembles the infective form of parasitic nematodes. Generally, animals that failed to complete a molt also ceased to develop, but they would occasionally escape old cuticle after several hours, only to become trapped again at the next molt, as observed in *qhg-1(RNAi)* larvae.

Reproductive Phenotype

While the majority of Mlt larvae arrest development and die, possibly as a consequence of starvation, Mlt animals trapped in cuticle during the L4-to-adult transition occasionally produced a limited number of progeny. This was observed in *qhg-1(RNAi), nhr-23(RNAi),* and *mlt-13(RNAi)* animals.

Phenotype Associated with Secretory Pathway Defects

RNAi against many genes known to function in the secretory pathway, such as the worm orthologs of the vesicle coat proteins SEC-23 and B-COP, disrupted molting (Table 2). Those secretory pathway components that gave a Mlt phenotype when inactivated by RNAi are listed in Table 2. The genes are listed by *C. elegans* cosmid name and open reading frame number. Homology searches using the blast algorithm and information available at wormbase (www.wormbase.org), a central repository of data on *C. elegans,* were used to identify the function of encoded proteins.

Table 2 RNAi against Secretory Pathway Components Produced Molt Defects

Gene	ORF	Molecular Function or Identity
nsf-1	H15N14.1	vesicular fusion; like human NSF
rab-5	F26H9.6	ras superfamily GTPase
tfg-1	Y63D3A.5	part of COPII complex; vesicle trafficking
snf-7	C56C10.3	vacuolar sorting
sar-1	ZK180.4	GTP-binding protein
arf-3	F57H12.1	GTP-binding protein
rab-1	C39F7.4	ras-family
sec-23	Y113G7A.3	COPII complex vesicular transport
dpy-23	R160.1	Clathrin adaptor complexes medium chain 7x
dyn-1	C02C6.1	dynamin family 8X
mlt-69	E03H4.8	beta coatomer-like
mlt-70	F59E10.3	Clathrin adaptor complex small chain
mlt-71	K12H4.4	signal peptidase
mlt-72	D1014.3	alpha-SNAP, NSF attachment protein
mlt-73	C13B9.3	clathrin adaptor
mlt-74	F43D9.3	sec1 family
	F41C3.4	homolog of *got-1* (GenBank Acc. No. NM_062446)
	F38A1.8	SRP-54 (GenBank Acc. No. NM_171254)

Interestingly, the bodies of animals undergoing RNAi against secretory pathway genes tended to disintegrate over time, distinguishing them from other Mlt larvae. The isolation of sixteen secretory pathway genes in a screen for larvae unable to molt indicated that a functional secretory pathway is needed

either to generate new cuticle or to export enzymes that allow larvae to break free of the old cuticle.

Larval Arrest Phenotypes

RNAi against genes shown in Table 3A produced molting defects in less than five percent of larvae, and also produced an early larval arrest phenotype (i.e., arrest in the L1 or L2 stage) in the majority of animals. RNAi against genes shown in Table 3B produced molting defects in 10% or less of larvae. This list identifies the target genes by *C. elegans* cosmid name and open reading frame number. Homology searches using the blast algorithm and information available at wormbase (www.wormbase.org), a central repository of data on *C. elegans,* were used to identify the function of encoded proteins.

Table 3A RNAi Produced Molt Defects in Less than 5% of Exposed Larvae

Gene	ORF	Molecular Function/Protein Domains
rpl-23	B0336.10	ribosomal protein
rps-0	B0393.1	ribosomal protein
rpl-14	C04F12.4	ribosomal protein L14
rps-3	C23G10.3	ribosomal protein
rps-10	D1007.6	ribosomal protein
rps-23	F28D1.7	ribosomal protein
rpn-7	F35H10.4	ribosomal protein
rps-21	F37C12.11	ribosomal protein
rps-14	F37C12.9	ribosomal protein
rps-11	F40F11.1	ribosomal protein
rps-22	F53A3.3	ribosomal protein
rps-16	T01C3.6	ribosomal protein
rps-19	T05F1.3	ribosomal protein S19
rpl-18	Y45F10D.12	ribosomal protein
mlt-75	C09F12.1	secretory protein
mlt-76	C09H10.2	Forkhead-associated (FHA) domain
mlt-77	C17H12.14	ATPase
mlt-78	C37C3.2	Domain found in IF2B/IF5 2x
mlt-79	D2085.1	
mog-5	EEED8.5	RNA helicase DEAD/DEAH box helicase
mig-10	F10E9.7	PH domain
mlt-80	F19F10.9	
mlt-81	F28F8.5	
mlt-82	F32D1.2	ATP synthase epsilion chain

vha-5	F35H10.4	H+ ion transport V-type ATPase 116kDa subunit family
mlt-83	F41E7.1	TM G-protein beta WD-40 repeats
mlt-84	F42A8.1	TGFB path
mlt-85	F54B3.3	AAA ATPase
mlt-86	F55A3.3	general chromatin factor
mlt-87	F56F3.5	Ribosomal protein S3A
mlt-88	H06I04.4a	4 ubiquitin domains, CH2 Zinc finger
mlt-89	K06A4.6	
mlt-90	K10D6.1	GABA receptor beta subunit
mlt-91	R06A10.1	
mlt-92	T07D10.1	transmembrane protein
lin-29	Y17G7A.2	lin-29
mlt-93	Y23H5A.7	aminoacyl-tRNA synthetase
vha-11	Y38F2AL.3	ATPase
	Y41D4B.21	
	Y41D4B.5	ion channel protein
	Y45F10B.5	
	Y55H10A.1	Cadherin
	ZK1236.3	
	ZK265.5	
	ZK265.6	G-protein coupled receptor
	ZK652.1	small nuclear ribonucleoprotein

Table 3B Gene inactivations that cause molting defects in 10% or less of larvae

Gene	ORF	Accession #	Molecular Identity
	B0348.1	refINM_070727	nematode-specific protein family
cle-1	C09F12.1	refINM_077446	claudin-like
	C23F12.1	refINM_077179	endothelial actin-binding protein repeats
	C37C3.2	gbIU64857	domain found in IF2B/IF5
	CD4.4	refINM_072073	coiled 4-coil domain
pas-6	CD4.6	refINM_072071	proteosome subunit
cdc-5	D1081.8	refINM_059902	myb-like DNA binding domain
pyr-1	D2085.1	refINM_063437	glutamine-dependent carbamoyl-phosphate synthase
mog-5	EEED8.5	refINM_062618	RNA helicase DEAD/DEAH box helicase
	F19F10.9	refINM_072551	SART-1 family
	F28F8.5	refINM_074471	coiled 4-coil domain, nematode specific
vha-5	F35H10.4	refINM_068998	H+ trans. V-type ATPase
	F25B4.6	refINM_072095	hydroxymethylglutaryl-coenzyme A synthase
elo-5	F41H10.7	refINM_068392	fatty acid elongation
	F42A8.1	refINM_063590	signal sequence, nematode specific
rpn-7	F49C12.8	refINM_069231	proteasome regulatory particle
	F53G12.4	refINM_058282	coiled 4-coil domain, nematode specific
	F54B3.3	refINM_063809	AAA ATPase
	F55A3.3	refINM_060420	metallopeptidase family M24
stc-1	F54C9.2	refINM_063407	truncated HSP
	H04M03.4	refINM_068483	novel
ubl-1	H06I04.4	refINM_171089	4 ubiquitin domains, CH2 Zinc finger
ceh-6	K02B12.1	refINM_059903	homeobox
	K06A4.6	refINM_073045	zinc metalloprotease like
slu-7	K07C5.6	refINM_073260	splicing factor
lag-1	K08B4.1	refINM_171350	transcriptional regulator
	R06A10.1	refINM_05841	ER membrane protein, nematode specific
kin-2	R07E4.6	refINM_07659	cAMP-dependent protein kinase
chp-1	R10E11.1	refINM_066760	CBP/p300 homolog
	T06D8.6	refINM_064002	cytochrome c o l heme lyase
	T19B10.2	refINM_073447	coiled coil domain, nematode specific
vha-4	T01H3.1	refINM_063258	vacuular proton ATPase, V0 proteolipid subunit C.
	T07D10.1	refINM_060791	signal peptide, nematode specific
crs-1	Y23H5A.7	refINM_058612	cysteinyl tRNA Synthetase
vha-11	Y38F2AL.3	refINM_067786	vacuolar H+ ATPase
vha-3	Y38F2AL.4	refINM_067787	vacuolar H+ ATPase
	Y45F10B.5	refINM_070216	transmembrane domains, nematode-specific
	Y55H10A.1	refINM_067931	H+ lysosomal ATPase like
sca-1	K11D9.2	refINM_066984	Sarco-Endoplasmic Reticulum Calcium ATPase
	ZK1236.3	refINM_066460	nematode specific
snr-5	ZK652.1	refINM_066307	small nuclear ribonuclear protein Sm F
rpl-23	B0336.10	refINM_065830	ribosomal protein
rps-0	B0393.1	refINM_065577	ribosomal protein
rpl-14	C04F12.4	refINM_060175	ribosomal protein L14
rps-3	C23G10.3	refINM_065948	ribosomal protein
rps-10	D1007.6	refINM_058997	ribosomal protein
rps-23	F28D1.7	refINM_069964	ribosomal protein
rps-21	F37C12.11	refINM_066178	ribosomal protein
rps-14	F37C12.9	refINM_066171	ribosomal protein
rps-11	F40F11.1	refINM_069785	ribosomal protein
rps-22	F53A3.3	refINM_065080	ribosomal protein
rpl-15	K11H12.2	refINM_066422	ribosomal protein
rps-16	T01C3.6	refINM_074289	ribosomal protein
rps-19	T05F1.3	refINM_060154	ribosomal protein S19
rpl-18	Y45F10D.12	refINM_070254	ribosomal protein
rps-1	F56F3.5	refINM_065509	ribosomal protein S3A
	C09H10.2	refINM_063974	ribosomal protein L44
	Y41D4B.5	refINM_067714	ribosomal protein S28e

The Mlt phenotype was observed after several days of exposure to dsRNA. Table 3A includes genes that encode ribosomal proteins that are likely to be required for larval growth and development, and are unlikely to be specifically required for molting. Table 3A also includes genes that are likely to function in neurons that regulate ecdysis. RNAi against neuroendocrine genes is expected to be relatively ineffective, given that neuronal genes are often refractory to RNAi. Nonetheless, such neural control genes are expected to be conserved among Ecdysozoans and therefore represent targets for the development of nematicides and insecticides. Neuronal *mlt* genes are inactivated in relatively few larvae exposed to dsRNA-expressing-bacteria.

Improved methods of RNAi are expected to identify additional *mlt* genes that function in the neuroendocrine regulation of molting. For example, the use of mutants that show enhanced RNAi, such as nematodes having a mutation in *rrf-3* (Simmer et al., *Curr Biol.* 12:1317, 2002) may increase the sensitivity of the RNAi-based screen for *mlt* genes. Nematodes having an *rrf-3* mutation may be screened using the methods described herein to identify new *mlt* genes. RNAi clones that disrupt molting only in hypersensitive strains likely act in neuroendocrine signaling pathways common to all Ecdysozoans (e.g., flies and nematodes). Drugs that targeted such proteins would be expected to disrupt molting in most Ecdysozoans, while having no adverse side effects on humans.

Pleiotropic Phenotypes

Pleiotropic phenotypes were associated with RNAi against sixteen open reading frames identified in the Mlt screen (e.g., F56C11.1 (*DuOx),* F53G12.3, F55A3.3, F18A1.3 (*lir-1),* ZK430.8, F41C3.4, Y48B6A.3, K07D8.1 (*mup-4),* W01G6.3, F57B9.2, K08B4.1 (*lag-1),* F49C12.12, F38H4.9, F25B4.6, ZK262.8, M162.6, ZK1073.1).

Conservation of *mlt* Genes

Table 4A shows the conservation of a subset of *mlt* genes across phylogeny, identifying the RNAi target genes by *C. elegans* cosmid name and open reading frame number, and their orthologs in *Drosophila melanogaster* (Dm), *Homo sapiens* (Hs), and *Saccharomyces cerevisiae* (Sc) by Genbank accession number and blast score. DNA sequences corresponding to the *mlt* genes of interest were retrieved from the repositories of sequence information at the NCBI website (http://www.ncbi.nlm.nih.gov/) or at wormbase (www.wormbase.org). The DNA sequence was then used for standard translating blast [tBLASTN] searching using the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). For each *mlt* gene, Table 4A identifies the Genebank accession number and blast score for the top blast hit from *Drosophila melanogaster* (Dm), *Homo sapiens* (Hs), and *Saccharomyces cerevisiae* (Sc). The DNA sequence corresponding to the top ortholog candidate produced by tblastn was retrieved from Genbank (http://www.ncbi.nlm.nih.gov/) and used for a BLASTx search of *C. elegans* proteins using the wormbase site (http://www.wormbase.org/db/searches/blast). In one preferred embodiment, conservation of the *mlt* gene in flies or humans was indicated when the BLASTx search produced the starting MLT protein as the top score. These most highly conserved sequences are shaded in deep color in Table 4A. All other related sequences are shaded with lighter color. These methods provided for the identification of orthologs of *C. elegans mlt* genes (Tables 1A, 1B, 4A-4D, 7 and 8) revealed by our RNAi analysis. An ortholog is a protein that is highly related to a reference sequence. One skilled in the art would expect an ortholog to functionally substitute for the reference sequence. Tables 4A and 7 list exemplary orthologs by Genbank accession number and blast score.

Table 4A Conserved *mlt* Genes

Gene	*D. melanogaster*		*H.sapiens*		*S. cerevisiae*
C09G5.6	gb|J02727	1E-12	ref|NM_033150	3E-16	
C17G1.6	ref|NM_079763	9E-24	ref|NM_012465	4E-29	
C23F12.1			ref|NM_001456	8E-47	
C34G6.6	ref|NM_143476	1E-13			
F08C6.1	ref|NM_137449	6E-36	gb|AF156100	6E-54	
F09B12.1					
F16B4.3					
F18A1.3					
F45G2.5					
F49C12.2	gb|AY118852	2E-09			
F53B8.1					
H04M03.4					
H19M22.1			gb|U42594	9E-11	
K07D8.1	gb|M16152	8E-44	gb|BC010444	3E-56	
M6.1	ref|NM_057268	8E-19	gb|M14144	7E-22	
M88.6	ref|NM_139674	2e-27	ref|NM_003667	1E-22	
T05C12.10	gb|L02793	6E-12	gb|BC033507	4e-09	
W01F3.3	gb|AY060635	4E-53	gb|J03225	6E-26	
W08F4.6					
Y111B2A.14					
ZK262.8					
ZK270.1	gb|AC008339	3E-24	emb|AL355493	9E-18	
ZK430.8	ref|NM_079167	E-103	dbj|D86983	e-103	
C01H6.5	gb|M90806	3E-71	emb|Y08639	7E-51	
F11C1.6	ref|NM_079419	3E-44	gb|AF124247	6E-42	
F52B11.3	ref|NM_080092	5E-32			
ZK686.3	gb|AY075331	2E-73			
C42D8.5	ref|NM_057698	2E-56	emb|X16295	4E-67	
F29D11.1	ref|NM_132335	0	ref|NM_004525	0	
F53G12.3	ref|NM_134871	0	ref|NM_014080	0	
F56C11.1	gb|AAF51201	0	ref|NM_014080	0	
F57B9.2	ref|NM_136653	e-132	ref|XM_168026	0	
K08B4.1	ref|NM_057520	e-134	gb|L07872	1E-126	
T01C3.1	ref|NM_080132	4E-24	gb|AF195765	2E-22	
Y37D8A.10	gb|AY094832	4E-25	ref|NM_014752	2E-26	
ZC101.2	emb|AJ487018	1E-160	gb|M85289	e-142	
ZK783.1	ref|NM_080072	3E-53	ref|XM_034890	5E-55	
ZK115.1	emb|AJ011925	0	ref|NM_000445	1E-52	
CD4.4			emb|AL834261	1E-12	
F20G4.1			ref|NM_015909	4E-10	

F54A5.1			ref| NM_024567	1E-17		
K04F10.4	ref|NM_078644	e-155	ref|NM_138319	e-145	gb| M22870	1E-
D1054.15	ref|NM_132550	e-161	ref| NM_002669	e-151	emb|Z73507	e-1
F25B4.6	ref|NM_079972	2E-83	emb|X83618	4E-86	emb|X96617	2E-
F33A8.1	gb|AY089504	e-155	ref|XM_034594	e-153	emb| Z73063	7E-
F38H4.9	emb|X78577	1E-167	ref|NM_004156	1E-70	emb|X56262	1E-1
F40G9.1	gb|AY118647	3E-13	ref| NM_002814	3E-24	Z73017	1E-
F41C3.4	gb| AY071265	4E-23	gb|AF151899	5E-25	emb|CAA56801	2E-
F41H10.7	ref|NM_140652	2E-35	ref|NM_152310	5E-25	gb|AF011409	5.00E
F54C9.2	ref|NM_078577	1E-63	ref| NM_006948	2E-81	emb| X12926	6E-
M03F8.3	emb|X58374	0	ref| NM_016652	0	emb| Z73289	e-1
R05D11.3	ref|NM_134578	1E-29	gb|BC002348	7E-20	gb|AAB64542	9e-
R07E4.6	gb|AY058709	e-117	M65066	e-115	gb| M15756	2E-
T01H3.1	gb|AY060235	3E-50	ref|XM_001463	3E-49	gb|M35294	6E-
T14F9.1	gb|AY052122	e-136	ref|NM_015941	e-132	dbj|D13916	1E-
T23F2.1	AY060893	2E-33	ref| NM_033087	5E-38	emb| X87947	1E-
T27F2.1	gb|AY113364	e-124	U51432	e-125	emb| X62577	2E-
Y48B6A.3	ref|NM_135238	0	ref| NM_012255	0	gb| S61567	e-1
ZK970.4	ref|NM_057621	1E-34	ref|NM_004231	7E-35	gb|U12786	3E-
W09B6.1	ref|NM_136498	1 e-164	emb| X68968	1 e-169	emb| Z22558	1E-

Key for Table 4A

D. melanogaster ortholog
D. melanogaster similar protein

Human ortholog
Human similar protein

Yeast ortholog
Yeast similar protein

Table 4B lists *C. elegans* genes and Drosophila and human orthologs identified using a tblastn search.

Table 4B Selected gene inactivations associated with molting defects

Category	Gene	ORF	Molecular Identity	SP[a]	Related Genes[b] Dm	Hs
Novel	*nlt-12*	W08F4.6		Y		
	nlt-13	F09B12.1	MAM domains	Y		
	pan-1	M88.6	leucine-rich repeats	Y	CG7509	GPR49
		F49C12.12		Y	GM16138	
		Y37D8A.10		Y	CG175	KIAA0102
		C37C3.3	DUF279	Y	CG8055	C20orf178
		ZK686.3		Y	CG783	N33
		F20G4.1				NAG
Proteases	*nlt-24*	C17G1.6	Astacin metalloprotease	Y	tolloid (tld)	TLL2
	nlt-21	C26C6.3	Astacin metalloprotease	Y	tolkin (tok)	TLL2
	acn-1	C42D8.5	Angiotension converting enzyme	Y	Ance	ACE
	nlt-20	F08C6.1	ADAM/reprolysin metalloprotease	Y	Sema-5c	hemicentin
	bli-4	K04F10.4	subtilase family serine protease	Y	Fur2	PACE 4
Protease Inhibitors	*nlt-17*	F45G2.5	pancreatic trypsin inhibitor domain	Y		
	nlt-18	W01H3.3	pancreatic trypsin inhibitor domains	Y	Ppn	TFPI
	nlt-19	B0024.14	serine protease inhibitor	Y	cv-2	CRIM1
Peroxidases		F53G12.3	animal haem peroxidase; gp91/phox1	Y	CG3131	DUOX2
	DuOx	F56C11.1	animal haem peroxidase; gp91/phox1	Y	CG3131	DUOX1/2
	nlt-11	ZK430.8	animal haem peroxidase; SbTk domain	Y	Pxn	KIAA0230
ECM	*nlt-14*	C34G6.6	PAN domains, ZP domain	Y	CG7802	
	nlt-15	F52B11.3	PAN domains, ZP domain	Y	nompA	
	nlt-16	ZK783.1	ECM microfibril component	Y	CG33196	FBN1
	bli-1	C09G5.6	cuticle collagen	Y	procollagen IV	COL2A1
Sterol-sensing domain	*lrp-1*	F29D11.1	LDL receptor related (megalin)	Y	CG12139	LRP2
	ptr-4	C45B2.7	patched family	Y	CG11212	na
	ptr-23	ZK270.1	patched family	Y	CG11212	na
muscle-hypodermis	*unc-52*	ZC101.2	basement mebrane proteoglycan	Y	trol	HSPG2
	let-805	H19M22.2	myotactin, fibronectin type III	Y	sdk	FLJ00154
	nmp-4	K07D8.1	EGF domains, SEA domain	Y	Notch	MATN2
Transcription factors & Co-factors	*nhr-23*	C01H6.5	nuclear hormone receptor		DHR3	ROR-beta
	nhr-25	F11C1.6	nuclear hormone receptor		ftz-F1	B1F2
	ltr-1	F18A1.3	transcription factor like lin-26			
	skp-1	T27F2.1	SKIP/SNW domain, co-repressor		Bx42	Skip
		F57B9.2	CCR4-Not complex, basal regulator		CG1884	CG1884
		F10C1.5	DSX DNA binding domain		dmrt99B	DMRTA2
Signaling		Y41D4B.10	Delta-serrate ligand precursor	Y	Delta	delta-4
	qhg-1	T05C12.10	hedgehog-like, hint module	Y	hh	
		F38H4.9	serine/threonine phosphatase		PP2	PPP2CB
		F53B8.1	Hu PLeC1 orthologue; plectin			
		T01C3.1	WD domain, G-beta repeats		l(2)dll	L2DFL
Others		Y48B6A.3	5'-3' exonuclease domain		CG10354	XRN2
		D1054.15	WD domains, G-beta repeats		CG1796	FLRG1
	ran-4	R05D11.3	nuclear import		Ntf2	Nlf2
		W09B6.1	acetyl-Coenzyme A carboxylase		CG11198	ACACA
		M03F8.3	Half-A-TPR repeats, RNA splicing		cm	CRNKL1

Figure 4B Legend: Top hits from tblastn searches of the human or fly genome using the predicted *C. elegans* gene product. Dark shading indicates that a blastx search with the predicted human or fly protein uncovered the corresponding *C. elegans* protein as the top-scoring match in *C. elegans*, identifying potential orthologs. Y indicates the presence of a secretory signal peptide (SP) in the predicted gene product.

Table 4C identifies genes whose inactivation disrupts molting and related genes in other species.

Table 4C *C. elegans* genes that disrupt molting and their counterparts in other species

Table S1. Genes whose inactivation disrupts molting and related genes in other species

Category	Gene	ORF	Accession #	Molecular Identity/Domains	SP	Related Genes								
						Drosophila melanogaster			Homo sapiens			Saccharomyces cerevisiae		
						Gene	Accession #	E value	Gene	Accession #	E value	Gene	Accession #	E value
Novel	*mlt-12*	W08F4.6	ref|NM_061358		Y									
	mlt-13	F09B12.1	ref|NM_078111	MAM domains	Y									
	pan-1	M88.6	ref|NM_065523	lecuine-rich repeats	Y	CG7509	ref|NM_139674	2e-27	GPR49	ref|NM_003667	e-22			
		F49C12.12	ref|NM_069234		Y	GM16138	gb|AY118852	e-10	HSPC254	gb|AF161372	8e-6			
		Y37D8A.10	ref|NM_067275		Y	CG175	ref|NM_132495	4e-25	KIAA0102	ref|NM_014752	2e-26			
		C37C3.3	ref|NM_072621	DUF279	Y	CG8055	ref|XM_080858	e-53	C20orf178	ref|NM_176812	6e-54	SNf7	gb|L09751	8e-23
		ZK686.3	ref|NM_066290		Y	CG783	ref|NM_135360	2e-73	N33	gb|BC010370	2e-75			
Proteases		F20G4.1	ref|NM_059784						NAG	ref|NM_015909	2e-10			
	mlt-24	C17G1.6	ref|NM_077268	Astacin metalloprotease	Y	tolloid (tld)	ref|NM_079763	9e-24	TLL2	ref|NM_012465	4e-29			
	mlt-21	C26C6.3	ref|NM_059708	Astacin metalloprotease	Y	tolkin (tok)	ref|NM_057531	4e-26	TLL2	ref|NM_012465	7e-27			
	acn-1	C42D8.5	ref|NM_076466	Angiotension converting enzyme	Y	Ance	ref| NM_057698	2e-56	ACE	emb|X16295	4e-67			
	mlt-20	F08C6.1	ref|NM_076885	ADAM/reprolysin metalloprotease	Y	Sema-5c	ref|NM_137449	6e-36	hemicentin	gb|AF156100	6e-54			
	bli-4	K04F10.4	ref|NM_059427	subtilase family serine protease	Y	Fur2	ref|NM_078644	e-155	PACE 4	ref|NM_138319	e-145	KeX2	gb| M22870	e-81
Protease Inhibitors	*mlt-17*	F45G2.5	ref|NM_067371	pancreatic trypsin inhibitor domain	Y									
	mlt-18	W01F3.3	ref|NM_075592	pancreatic trypsin inhibitor domains	Y	Ppn	ref|NM_176574	4e-53	TFPI	gb|J03225	6e-26			
	mlt-19	B0024.14	ref|NM_073255	serine protease inhibitor	Y	cv-2	ref|NM_080070	4e-16	CRIM1	ref|NM_016441	e-120			
Peroxidases		F53G12.3	ref|NM_058283	Animal haem peroxidase; gp91/*phox1*	Y	CG3131	ref|NM_134871	0	DUOX2	ref|NM_014080	0			
	DuOx	F56C11.1	ref|NM_058285	Animal haem peroxidase; gp91/*phox1*	Y	CG3131	ref|NM_134871	0	DUOX1/2	ref|NM_014080	0			
	mlt-11	ZK430.8	ref|NM_062376	Animal haem peroxidase; ShTk domain	Y	Pxn	ref|NM_079167	e-103	KIAA0230	dbj| D86983	e-103			
ECM	*mlt-14*	C34G6.6	ref|NM_059305	PAN domains, ZP domain	Y	CG7802	ref| NM_143476	e-13						
	mlt-15	F52B11.3	ref|NM_070298	PAN domains, ZP domain	Y	nompA	ref|NM_080092	5e-32						
	mlt-16	ZK783.1	ref|NM_066269	ECM microfibril component	Y	CG33196	ref|NM_175960	4e-58	FBN1	ref|NM_000138	5e-55			
	bli-1	C09G5.6	ref|NM_063910	cuticle collagen	Y	procollagen IV	gb|J02727	e-12	COL2A1	ref|NM_033150	3e-16			
Sterol-sensing domain	*lrp-1*	F29D11.1	ref|NM_059726	LDL receptor related (megalin)	Y	CG12139	ref|NM_132335	0	LRP2	ref| NM_004525	0			
	ptr-4	C45B2.7	ref|NM_076612	patched family	Y	CG11212	ref|NM_136365	1e-55	na	emb|AL355493	3e-37			
	ptr-23	ZK270.1	ref|NM_061202	patched family	Y	CG11212	ref|NM_136365	2e-46	na	emb|AL355493	9e-18			
muscle-hypodermis	*unc-52*	ZC101.2	ref|NM_064645	basement mebrane proteoglycan	Y	trol	emb| AJ487018	e-160	HSPG2	gb|M85289	e-142			
	let-805	H19M22.2	ref|NM_065198	myotactin, fibronectin type III	Y	sdk	ref|NM_134315	2e-44	FLJ00154	dbj|AK074083	4e-54			
	lfc-2	M6.1	ref|NM_075732	intermediate filament protein		Lam	ref|NM_057268	8e-19	vimentin	gb|M14144	7e-22			
	ifa-2	W10G6.3	ref|NM_078247	intermediate filament protein		Lam	ref|NM_057268	4e-49	LMNB2	ref|NM_032737	5e-61	INT1	gb|L03188	e-13
	mup-4	K07D8.1	ref|NM_066244	EGF domains, SEA domain	Y	Notch	ref|NM_057511	7e-47	MATN2	ref|NM_030583	1e-56			
Transcription factors & Co-factors	*nhr-23*	C01H6.5	ref|NM_059638	nuclear hormone receptor		DHR3	gb|M90806	3e-71	ROR-beta	emb|Y08639	7e-51			
	nhr-25	F11C1.6	ref| NM_077761	nuclear hormone recptor		ftz-F1	ref|NM_079419	3e-44	B1F2	gb|AF124247	6e-42			
	lir-1	F18A1.3	emb|AJ130959	transcription factor like *lin-26*										
	skp-1	T27F2.1	ref|NM_073549	SKIP/SNW domain, co-repressor		Bx42	gb|AY113364	e-124	Skip	U51432	e-125	FUN20	emb| X62577	2e-16
		F57B9.2	ref|NM_066115	CCR4-Not complex, basal regulator		CG1884	ref|NM_136653	e-132	CG1884	ref|NM_136653	0	CDC39	emb|X70151	e-52
		F10C1.5	ref|NM_062737	DSX DNA binding domain		dmrt99B	ref|NM_079825	5e-31	DMRTA2	ref|XM_027162	2e-30			
Signaling		Y41D4B.10	ref|NM_067707	Delta-serrate ligand precursor	Y	Delta	ref|NM_169853	2e-05	delta 4	gb|AF279305	e-05			
	qhg-1	T05C12.10	ref|NM_063324	hedgehog-like, hint module	Y	hh	gb|L02793	6e-12		gb| BC033507	4e-09			
		F38H4.9	ref|NM_069846	serine/threonine phosphatase		PP2	emb|X78577	e-167	PPP2CB	ref|NM_004156	e-70	PPH22	emb|X56262	1e-141
		M03F4.7	ref|NM_076443	calcium binding, EF-hand family	Y	scf	ref|NM_058044	e-71	CALU	ref|NM_001219	5e-79			
		T01C3.1	ref|NM_074284	WD domain, G-beta repeats		l(2)dtl	ref|NM_080132	4e-24	L2DTL	gb|AF195765	2e-22			
ATPases		ZK970.4	ref|NM_063816	H+-trans. ATPase, F subunit family		Vha14	ref|NM_057621	e-34	ATP6V1F	ref|NM_004231	7e-35	VMA7	gb|U12786	3e-22
	vha-8	C17H12.14	ref|NM_068639	H+-trans. ATPase, E subunit family		Vha26	ref|NM_079513	e-69	ATP6V1E1	ref|NM_001696	e-55	VMA4	emb|Z75239	2e-27
		T14F9.1	ref|NM_076011	V-ATPase, H subunit		VhaSFD	ref|NM_165178	e-136	ATP6V1H	ref|NM_015941	e-132	VMA13	dbj|D13916	1e-26
Others	*pbs-5*	K05C4.1	ref|NM_061157	proteosome B-type subunit		Prosbeta5	ref|NM_143757	2e-53	LMP7C	gb|U17497	8e-51	PRG1	gb|M96667	1e-49
		D1054.15	ref|NM_073362	WD domains, G-beta repeats		CG1796	ref|NM_132550	e-161	PLRG1	ref|NM_002669	e-151	PRP46	emb|Z73507	e-102
		Y48B6A.3	ref|NM_064557	5'-3' exonuclease domain		CG10354	ref|NM_135238	0	XRN2	ref| NM_012255	0	TAP1	gb| S61567	e-114
	ran-4	R05D11.3	ref|NM_059921	nuclear import		Ntf-2	ref|NM_134578	e-29	Ntf2	gb|BC002348	7e-20	HFA1	gb|AAB64542	9e-17
		W09B6.1	ref|NM_061521	acetyl-Coenzyme A carboxylase a		CG11198	ref|NM_136498	e-165	ACACA	ref|NM_000664	1e-67	Acc1p	emb|Z22558	e-159
	let-858	F33A8.1	ref|NM_063962	nucampholin		CG12750	ref|NM_136033	e-155	KIAA1604	ref|XM_034594	e-153	YGR278w	emb| Z73063	7e-50
		M03F8.3	ref|NM_072146	Half-A-TPR repeats, RNA splicing		crn	emb|X58374	0	CRNKL1	ref|NM_016652	0	YLR117c	emb| Z73289	e-112
		T23F2.1	ref|NM_076531	glycosyltransferase		CG1291	ref|NM_139515	e-33	FLJ14511	ref|NM_033087	5e-38	ALG2	emb| X87947	e-28
	rme-8	F18C12.2	ref|NM_059821	DNAJ domain, endocytosis factor		CG8014	ref|NM_136623	0	KIAA0678	ref|XM_039828	0			e-4
		F40G9.1	ref|NM_064775	Ankryin repeats, cytoskeleton		CG5841	ref|NM_140569	3e-13	PSMD10	ref|NM_002814	3e-24	YGR232w	emb| Z73017	e-15

Table 4D shows homologs of selected *mlt* genes in parasitic nematode species

Table 82. Homologs of selected molting genes in parasitic nematode species

Gene	ORF	Related Genes (1)																				
		Onchocerca volvulus		*Brugia malayi*		*Strongyloides stercoralis*		*Strongyloides ratti*		*Ancylostoma ceylanicum*		*Ancylostoma caninum*		*Necator americanus*		*Ascaris suum*		*Haemonchus contortus*		*Dirofilaria immitis*		*Trichuris muris*	
		Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value	Accession #	E value
nhr-23	C01H6.3			dbj|AU000440																			
bli-1	C09G5.6	gb|BF482033	4E-19	gb|AI066836	3E-22	gb|BG226349	7E-25									gb|BM281749	2E-39						
mlt-24	C17G1.6			gb|AA471557	5E-17	gb|BG224501	9E-35	gb|BI741990	3E-39							gb|AW165858	1E-26						
mlt-21	C26C6.3					gb|BE224326	6E-32	gb|BI323632	6e-29			gb|BQ667369	3E-21	gb|BU089288	2E-29	gb|BQ835552	8E-41						
mlt-14	C34G6.6			gb|AA471404	2E-68	gb|BE579677	2E-53	gb|BI073876	1E-41	gb|CB276958	2E-36			gb|BU088646	2E-33	gb|BU965942	8E-38						
acn-1	C42D8.5															gb|BI782938	9E-52	gb|BF060055	4E-18				
	CD4.4																					gb|BG577864	4E-12
	D1054.15	gb|BE202350	9E-49																			gb|BM277122	6E-15
mlt-20	F08C6.1					gb|BE581131	2E-34					gb|BG232752	4E-77	gb|BU088714	1E-108								
mlt-13	F09B12.1	gb|AI665735	4E-10			gb|BG226227	1E-23																
nhr-25	F11C1.6			dbj|AU000440		gb|BE581104	1E-27																
lrp-1	F29D11.1			gb|AW055802	3E-38											gb|BM280724	6E-20					gb|BF169279	5E-11
	F38H4.9							gb|BG893946	3E-27														
	F40G9.1													gb|BU666328	1e-118	gb|BI1782814	8E-89						
mlt-17	F45G2.5											gb|CA341524	3E-37	gb|BG467849	3E-13	gb|BI594288	8E-29						
	F49C12.12					gb|BE029934	1E-15																
mlt-15	F52B11.3			gb|AA661399	4E-48	gb|BE580180	1E-73	gb|BG893830	7E-80	gb|BQ274691	1E-34	gb|BF250630	1E-22	gb|BU089096	9E-37							gb|BM174586	2E-19
	F53G12.3			gb|AA161577	1E-39																		
mc-1	F54C9.2											gb|AW735074	6E-64										
DuOX	F56C11.1			gb|AA161577	1E-39	gb|BG226148	1E-44																
	F57B9.2											gb|AW735074	6E-64										
	H04M03.4	gb|AA294602	5E-15	gb|AI710981	2E-27	gb|BG227443	2E-75	gb|BI397280	1E-61			gb|BU780997	6E-53										
	H19M22.2																						
bli-4	K04F10.4					gb|BE028912	7E-24	gb|BQ091197	2E-18			gb|BF250605											
mup-4	K07D8.1			gb|AI783143	1E-66	gb|BE223322	2E-23	gb|BI450575	3E-38			gb|BQ666394	2E-24	gb|BU087198	4E-14			gb|CA033722	1E-95				
	M03F4.7			gb|BM889340	1E-78	gb|BG226767	4E-85	gb|BI450741	2E-98														
lfe-2	M6.1	gb|AA917260	2E-19	gb|AW675831	8E-19	gb|BF014961	3E-28	gb|BI742464	8E-19			gb|BQ666411	1E-111			gb|BM319475	1E-103	gb|CA033609	1E-62				
pan-1	M88.6															gb|BM280603	1E-28	gb|BF060126	4E-25				
	T01C3.1																					gb|BM174670	8E-32
qhg-1	T05C12.10	gb|AW257707	1E-22			gb|BF014893	1E-34																
	T14F9.1					gb|BG226359	5E-70																
	T23F2.1					gb|BE579591	7E-75					gb|BM130242	4E-72	gb|BU086612	5E-65	gb|BI594547	4E-67						
ckp-1	T27F2.1																						
mlt-18	W01F3.3					gb|BE580288	3E-20	gb|BG893620	2E-19					gb|BU087096	1E-22								
mlt-12	W08F4.6	gb|BG310588	E-121	gb|BE753466	E-104	gb|BG227161	2E-84					gb|BM077795	2E-19	gb|BU666009	1E-21			gb|BF422862	9E-18			gb|BM174557	9E-21
	W09B6.1					gb|BE580061	8E-43	gb|BQ091288	7E-29														
lfe-2	W10G6.3	gb|BF199444	2E-62	gb|AW675831	2E-75	gb|BE224367	7E-43	gb|BI742464	8E-50			gb|BQ125044	2E-61	gb|BU666204	2E-15	gb|BI782124	2E-47						
	Y37D8A.10					gb|BE029374	1E-37									gb|BM280603	1E-84	gb|BF060126	5E-57	gb|BQ455787	1E-35	gb|BF049882	2E-69
	Y48B6A.3									gb|BQ288481	2E-59			gb|BU666155	9E-27			gb|BI595303	3E-68				
mo-52	ZC101.2	gb|AW980135	4E-62	gb|AI723671	5E-60	gb|BG227295	1E-49	gb|BI324097	6E-40														
ptr-23	ZK270.1	gb|BE202282	2E-11	gb|AW257677	3E-43	gb|BE579648	5E-54	gb|BI323571	3E-44			gb|BE352403	4E-19			gb|BI782862	1E-13	gb|BE496755	3E-99	gb|BQ454813	5E-58		
mlt-11	ZK430.8			gb|AI723670	8E-40	gb|BG227360	5E-72	gb|BI073673	5E-31			gb|BM130388	9E-98	gb|BU086563		gb|BM033843	7E-20						
	ZK686.3			gb|AA842318	8E-22	gb|BE581316	2E-48							gb|BG467473	6E-22			gb|BF423018	9E-74				

1 Top hits from tblastn searches with the predicted *C. elegans* gene product versus translated cDNAs isolated from the indicated species.

Table 4D (continued)

Table S2 (continued). Homologs of selected molting genes in parasitic nematode species

Gene	ORF	Related Genes: Trichinella spiralis Accession #	Trichinella spiralis E value	Toxocara canis Accession #	Toxocara canis E value	Globodera pallida Accession #	Globodera pallida E value	Globodera rostochiensis Accession #	Globodera rostochiensis E value	Meloidogyne arenaria Accession #	Meloidogyne arenaria E value	Meloidogyne incognita Accession #	Meloidogyne incognita E value	Meloidogyne javanica Accession #	Meloidogyne javanica E value	Meloidogyne hapla Accession #	Meloidogyne hapla E value	Heterodera glycines Accession #	Heterodera glycines E value	Pristionchus pacificus Accession #	Pristionchus pacificus E value	Parastrongyloides trichosuri Accession #	Parastrongyloides trichosuri E value	Ostertagia ostertagi Accession #	Ostertagia ostertagi E value
nhr-23	C01H6.5	gb|BG353339	3E-29																						
bli-1	C09G5.6					gb|BM415129	1E-20																		
mlt-24	C17G1.6	gb|BQ738378	2E-17																						
mlt-21	C26C6.3									gb|BI747415	2E-17									gb|BI500840	2E-23			gb|BG734092	1E-22
mlt-14	C34G6.6							gb|BM343299		gb|BI747765	2E-25											gb|BI451087	2E-34	gb|BG733933	6E-20
acn-1	C42D8.5	gb|BG520845	1E-15							gb|BI745765	4E-10			gb|BI744615	4E-44					gb|BI500192	2E-69	gb|BI451087	5E-35	gb|BG734159	9E-26
	CD4.4	gb|BG519941	6E-11							gb|BI501765	4E-41	gb|BM881559	8E-41	gb|BG735807	6E-38	gb|BM902335	9E-26			gb|AW114662	3E-39	gb|BI451241	6E-33		
	D1054.15	gb|BG520170	2E-28			gb|BM415102	4E-56													gb|AW097092	8E-24				
mlt-20	F08C6.1									gb|BI863068	2E-87									gb|AW115214	3E-29				
mlt-13	F09B12.1																								
nhr-25	F11C1.6																								
lrp-1	F29D11.1					gb|BM415763	E-119	gb|BM354985 gb|AW506417	2E-17 2E-86																
	F38H4.9															gb|BM901359	2E-43								
	F40G9.1													gb|BI744849	4E-79										
mlt-17	F45G2.5																					gb|BI863834	2E-69	gb|BQ097609	1E-104
	F49C12.12	gb|BQ543136	4E-17																						
mlt-15	F52B11.3							gb|AW506351	2E-36					gb|BI745272	3E-12	gb|BU094732	2E-30	gb|BF013515	2E-36			gb|BM513019	5E-30		
	F53G12.3															gb|BM952243	9E-71								
stc-1	F54C9.2																								
DuOX	F56C11.1																			gb|AW052295	1E-55				
	F57B9.2																								
	H04M03.4											gb|BM881751	3E-36												
	H19M22.2									gb|BI745690	1E-61					gb|BM902109	2E-56								
bli-4	K04F10.4	gb|BQ693113	1E-51					gb|BM343207 gb|AW506559	 8E-34																
mup-4	K07D8.1											gb|BM880593	9E-14			gb|BM901742	2E-20					gb|BI451155	2E-63	gb|BQ099039	5E-18
	M03F4.7			gb|BM966480	9E-90	gb|BM415425	1E-66					gb|BE238861	8E-38												
lfe-2	M6.1	gb|BG353660	5E-26	gb|BM965806	4E-16											gb|BM900690	2E-83			gb|AI986802	2E-52			gb|BM896658	3E-79
pan-1	M88.6									gb|BI746256	3E-15	gb|BQ613722	8E-25			gb|BQ836630								gb|BQ099825	7E-20
	T01C3.1													gb|BG735742	5E-14										
qhg-1	T05C12.10																								
	T14F9.1					gb|BM415082	2E-60	gb|BM345905	2E-73			gb|BQ613344	7E-47												
	T23F2.1													gb|BG735889	5E-65			gb|BF014612	2E-54						
zkp-1	T27F2.1											gb|BM880892	6E-65	gb|BI744669	3E-52	gb|BM883631	2E-57			gb|BI703617	4E-13	gb|BM513291	5E-69		
mlt-18	W01F3.3	gb|BQ692168	2E-8									gb|BM881774	8E-22	gb|BI142900	3E-44	gb|BM900937	1E-33								
mlt-12	W08F4.6									gb|BI746672	6E-31	gb|BM882536	6E-30	gb|BI745590	9E-18	gb|BM902581	5E-19								
	W09B6.1			gb|BM966530	1E-37																				
lfu-2	W10G6.3	gb|BG353660	6E-68	gb|BM965806	1E-52																	gb|BM346811	6E-89		
	Y37D8A.10							gb|BM344699	3E-78	gb|BI747934	8E-53	gb|BQ613497	1E-68			gb|BM901834	6E-66								
	Y48B6A.3									gb|BI747379	4E-30	gb|BM882772	4E-24					gb|BI396794	1E-29	gb|AW052236	7E-51	gb|BI322222	1E-43	gb|BM896621	6E-77
unc-52	ZC101.2			gb|BM965583	9E-59			gb|BM345416	3E-13													gb|BI744051	3E-29		
ptr-23	ZK270.1							gb|AW506417	1E-14			gb|BQ613494	3E-21			gb|BM901402	2E-44							gb|BQ457535	6E-52
mlt-11	ZK430.8	gb|BG353679	3E-28					gb|BM344825	7E-18	gb|BI746878	2E-12	gb|BE240858	6E-14			gb|BQ090105	2E-17			gb|AA193996	1E-62	gb|BM513799 gb|BI863807	1E-14 4E-31		
	ZK686.3					gb|AW505639	1E-32									gb|BM883419	1E-36	gb|BI396718	1E-27	gb|AW097184	9E-71				

mlt-26, which encodes the worm ortholog of *fibrilin-1*, is conserved in humans. The human gene is associated with Marfan syndrome. MLT-14 and MLT-15 are homologous to NompA, a component of specialized extracellular matrix (ECM) in flies (Chung et al., *Neuron* 29:415-28, 2001). Putative modification enzymes include MLT-24 and MLT-21, tolloid family metalloproteases that might direct cuticle assembly by processing procollagens or other ECM proteins, just as tolloid family members regulate vertebrate ECM formation, in part, by cleaving procollagen C-propeptides (Unsold et al. *JBC* 277:5596-602, 2002; Rattenholl et al., *JBC* 277:26372-8, 2002). MLT-17 and MLT-18 likely inhibit extracelullar proteases, since both proteins contain domains similar to BPTI, and a comparable ECM protein of *D. melanogaster* inhibits metalloproteinases *in vitro* (Kramerova et al., *Dev* 127:5475-85, 2000). Of three peroxidases essential for molting, one, DuOx, probably crosslinks cuticle collagens (Edens et al., *J. Cell Biol* 154:879-91, 2001). Together, these enzymes likely regulate the spatial and temporal dynamics of epithelial remodeling during molting, and regulation of the corresponding genes maytherefore ensure the orderly synthesis and breakdown of cuticle.

Neuroendocrine pathways regulate molting in arthropods, and likely also operate in nematodes. In insects, pulses of the steroid hormone 20-hydroxyecdysone trigger molting and metamorphosis, and the neuropeptide PTTH stimulates ecdysone synthesis in the prothoracic glands (Gilbert et al., *Ann. Rev. Entomol.* 47:883-916, 2002). The peptide hormone ETH drives behavioral routines essential for ecdysis (Park et al., Dev. 129:493-503, 2002; Zitnan et al., Science 271: 88-91, 1996), and the neuropeptide eclosion hormone (EH) triggers ETH secretion from epitracheal glands, in part. Environmental and 4 physiologic cues modulate secretion of PTTH, suggesting extensive neural input to the neuroendocrine secretions that govern molting (Gilbert et al., *Ann. Rev. Entomol.* 47:883-916, 2002).

In *C. elegans*, the requirement for two orphan nuclear hormone receptors, NHR- 23 and NHR-25, orthologous, respectively, to the ecdysone-responsive gene products DHR3 and Ftfz-F1 of *Drosophila melanogaster* (Kostrouchova Dev. 125:1617-26, 1998; Gissendanner et al., Dev Biol 221:259-72, 2000), implicates an endocrine trigger for molting, possibly derived from steroids. Consistently, molting requirescholesterol, the biosynthetic precursor of all steroid hormones (Yochem et al. Dev. 126:597-606, 1999). Further, molting of the nematode *Aphelenchus avenae* requires a diffusible signal from the anterior of the worm (Davies et al., *Int. J. Parasitol* 24:649-55, 1994), pointing to an endocrine cue. Ecdysone itself, however, is unlikely to serve as a nematode molting hormone, because orthologs of the ecdysone receptor components ECR and USP have not been identified in the fully-sequenced genome of *C. elegans* (Sluder et al., *Trends Genet* 17:206-13, 2001), and because ecdysteroids have not been detected in any free-living nematode (Chitwood, *Crit Rev Biochm Mol Biol* 34:273-84, 1999). Several genes uncovered in our screen encode signaling molecules and transcription factors that might transduce endocrine signals for molting between neurons and epithelial cells (Table 1A and Table 1B), such as QHG-1 (quahog), a protein with a C-terminal Hint domain like that found in hedgehog (Aspock et al., *Gen. Res.* 9:909-23, 1999), as well NHR-23 and NHR-25, both synthesized in epithelial cells (Kostrouchova et al., Dev 125:1617-26, 1998; Gissendanner, *Dev Biol* 221:259-72, 2000). The *mlt-12* or Y41D4B.10 genes might specify intercellular signals regulating molting, since the corresponding proteins contain secretory signal sequences, but lack transmembrane domains or motifs characteristic of ECM proteins. Moreover, dibasic sites in MLT-12 suggest proteolytic processing, while Y41D4B.10p resembles a delta/serrate ligand. ACN-1 is also predicted to function in the endocrine phase of molting, as the protein is 28% identical to human angiotensin converting enzyme (ACE), the peptide protease that cleaves angiotensin I to 5 angiotensin II. ACN-1 is

unlikely to catalyze proteolysis, because the active-sites residues of ACE are not conserved in the predicted ACN-1 protein. Nevertheless, ACN-1 could regulate production of a peptide molting hormone.

Twenty-three of the *mlt* genes identified herein (e.g., C09G5.6, C17G1.6, C23F12.1, C34G6.6, F08C6.1, F09B12.1, F16B4.3, F18A1.3, F45G2.5, F49C12.2, F53B8.1, H04M03.4, H19M22.2, K07D8.1, M6.1, M88.6, T05C12.10, W01F3.3, W08F4.6, Y111B2A.14, ZK262.8, ZK270.1, and ZK430.8) appear unique to nematodes since sequence orthologs of the corresponding proteins were not identified in *D. melanogaster* or *H. sapiens*, but were readily identified among the predicted products of cDNAs derived from parasitic nematode species that infect mammals and insects. For *mlt-12*, thirty-two different cDNAs (Table 7) isolated from a library of molting *O. volvulus* larvae, the parasite associated with African River Blindness, were found to be orthologous. Whereas many cDNAs matching *mlt-12* (e^{-121}) were found in a library from molting *O. volvulus* (Table 4C), a similar gene was not found in the fly or human genomes. Identifying genes essential for *C. elegans* molting enables the development of safe and effective nematicides that, for example, target gene products conserved only in nematodes. One attractive target is MLT-12, because the *mlt-12* gene is conserved and highly expressed at the molt in a parasitic nematode.

Molting proteases, like MLT-24, also represent attractive targets for the development of small molecule antagonists, given the success of drug development on protease targets for high blood pressure and HIV (Cvetkovic et al, 63:769-802, 2003). Moreover, pesticides that target molecular components of molting shared between arthropods and nematodes, such as the ECM proteins MLT-14 and MLT-15, are expected to harm only Ecdysozoans, and therefore be much less toxic to humans than current insecticides.

The methods of the invention are useful for treating or preventing an *O. volvulus* parasitic infection by inhibiting *O. volvulus mlt-12*. In one embodiment, an RNA *O. volvulus mlt-12* nucleic acid inhibitor is administered

to an infected person or to a person at risk of infection, for example, a person living in an area in which *O. volvulus* is endemic. This administration inhibits molting in *O. volvulus*, interrupts the life cycle of the causitive agent of African River Blindness, and treats or prevents an *O. volvulus* infection. Because there is no *mlt-12* human homologue, administration of a chemical compound or RNA nucleic acid inhibitor of *mlt-12* would be expected to produce few, if any, adverse human side effects.

Several of the *mlt* genes identified herein and presented in Table 4A were found in insects and nematodes, but not in yeast, suggesting that their protein products are good candidates to function in molting in all Ecdysozoans. In particular, *mlt-15*, which corresponds to F52B11.3, and ZK686.3 have orthologs in *Drosophila*, but homologous genes were not identified in other metazoans or yeast. Genes present in Ecdysozoans (e.g., *Drosophila*, *C. elegans* and other nematodes), but missing or divergent in non-molting organisms (e.g., chordate clade members, such as vertebrates), likely function in molt neuroregulatory pathways. Given that Ecdysozoans are distant from humans and are the only animals that molt, it is likely that *mlt* genes that are present only in Ecdysozoans can be inhibited with drugs or siRNAs that will not have adverse side effects in humans.

Regulation of *mlt* Gene Expression

To determine if the newly-identified *mlt* genes are periodically or continually expressed during larval development, gene fusions were generated in which GFP was expressed under the control of the *mlt-12*, *mlt-13*, *mlt-18*, *mlt-10*, *mlt-24*, and *acn-1* promoters. To shorten the half-life of the GFP fusion proteins to approximately one hour *in vivo*, a PEST sequence driving rapid protein degradation (Loetscher et al., *J. Biol. Chem.* 266:11213-20) was added to the end of the GFP open reading frame. The fusion genes were each microinjected into temperature-sensitive *pha-1(e2123)* mutant animals along with a *pha-1*(+) rescuing construct. Table 5 lists strains used in this study.

Table 5 Strains Used in *mlt* GFP PEST Expression

Strain	Genotype	Source	Reference
N2	wild-type	CGC	
GE24	*pha-1(e2123)* III	CGC	Granato et al., 1994
NL2099	*rrf-3(pk1426) II*	CGC	
GR1348	pha-1(e2123) mgEx646[P_{mlt-10}::GFP-PEST pha-1^+]	this study	
GR1349	pha-1(e2123) mgEx647[P_{mlt-12}::GFP-PEST pha-1^+]	this study	
GR1350	pha-1(e2123) mgEx648[P_{mlt-13}::GFP-PEST pha-1^+]	this study	
GR1351	pha-1(e2123) mgEx649[P_{mlt-18}::GFP-PEST pha-1^+]	this study	
GR1368	*pha-1(e2123) mgEx656* [*mlt-24::gfp-pest pha-1*]	this study	
GR1367	*pha-1(e2123) mgEx654* [*acn-1::gfp-pest pha-1*]	this study	
GR1348	*pha-1(e2123) mgEx657* [*mlt-10::gfp-pest pha-1*]	this study	
GR1387	*pha-1(e2123) mgEx659* [*mlt-13::gfp pha-1*]	this study	

Table 6 lists the primers used to construct the *mlt* GFP-PEST fusion genes.

Table 6: *mlt* Gene Primers

Table S6. Primers for construction of GFP fusion genes

Gene	Primer U1	Primer U2
mlt-12	5' TAAATTTTGGAGGGTCTCGGC 3'	5' GGAAAAACGACACGACTATGG 3'
mlt-13	5' TTAATTGCCGCGCAAAATGCG 3'	5' ATGCGACGAAATCACTACTCGG 3'
mlt-18	5' GCGATGGAGTACCACTTGGCGATTTTTGG 3'	5' GCTAGAAATGGGTGAAATCGGTCTTCCGG 3'
acn-1	5' ACCGTGATTGGACTGTTTTCAGTGCACC 3'	5' ACCGTGATTGGACTGTTTTCAGTGCACC 3'
mlt-24	5' GCTTTGAACCCGCAGACACTAAGATTGG 3'	5' TGAACTGACGAAACTGGGAGGATAACCG 3'
mlt-10	5' GTTAGCCTTCCAACCTGAATAGAGAACAGG 3'	5' GTTAGCCTTCCAACCTGAATAGAGAACAGG 3

Gene	Primer R1*	Primer R1
mlt-12	5' TTTAAAATCAAATTTCTCAGGTAATG-R1 3'	5' R2-CATTACCTGAGAAATTTGATTTTAAA 3'
mlt-13	5' TATCCGACCACACTACCATCAGAATG-R1 3'	5' R2-CATTCTGATGGTAGTGTGGTCGGATA 3'
mlt-18	5' AATTCCTATCAGTTGTCGGGTAATG-R1 3'	5' R2-CATTACCCGACAACTGATAGGAATT 3'
acn-1	5' TTATTTATAGTTGTTTTTCAGATG-R1 3'	5' R2-CATCTGAAAAACAACTATAAATAA 3'
mlt-24	5' TCTTGATGTTCTATTTTGCAGAATG-R1 3'	5' R2-CATTCTGCAAAATAGAACATCAAGA 3'
mlt-10	5' GTAATAAATTTTGGCAATAAATCATG -R1 3'	5' R2- CATGATTTATTGCCAAAATTTATTAC 3'

* R1 refers to the sequence 5' CGGGATTGGCCAAAGGACCCAAAG 3'
R2 refers to the sequence complementary to R1

For each reporter, genomic DNA isolated from N2 worms was amplified using primers A1 (SEQ ID NOs:1-3) and FL (SEQ ID NOs:10-12), while DNA from pAF207 was amplified using primers FU (SEQ ID NOs:7-9) and CAW31 (5' GCCGCATAGTTAAGCCAGCC 3' (SEQ ID NO:13), (Wolkow et al., *Science* 290: 147-50, 2000), using high-fidelity Taq. The EXPAND LONG TEMPLATE PCR SYSTEM (Roche Molecular Biochemicals), a kit containing PCR reagents, was used for all reactions.

The two PCR products were annealed and the resulting polynucleotide amplified using primers A2 (SEQ ID NO:4-6) and CAW32 (5' CCGCTTACAGACAAGCTGTGACCG 3') (SEQ ID NO:16). To add the PEST sequence to the C-terminus of GFP, nucleotides 1399-1524 of pd1EGFP-N1 (Invitrogen) were inserted into pPD95_81 (provided by A. Fire) between the last coding codon and the stop codon of GFP. This generated vector pAF207. The reporter constructs fpAF15, fpAF9, and fpAF12 correspond, respectively, to *Pmlt-12::GFP-PEST*, *Pmlt-13::GFP-PEST*, and *Pmlt-18::GFP-PEST*. In Table 6, R_1 refers to the DNA sequence: 5' CGGGATTGGCCAAAGGACCCAAAG 3'(SEQ ID NO:14) and R_2 refers to the DNA sequence 5' CTTTGGGTCCTTTGGCCAATCCCG 3'(SEQ ID NO:15). To generate the extrachromosomal arrays mg647, mg648, and mg649, respectively, fpAF15, fpAF9, and fpAF12 were purified by gel electrophoresis and then microinjected into *pha-1(e 2123)* mutant animals along with the *pha-1*$^+$ plasmid pBX at 3ng/ul (Granato et al., *Nucleic Acids Res.*, 22: 1762-3, 1994) and pBS DNA bringing the final DNA concentration to 100 ng/ul. Transgenic lines were recovered as described (Granato et al., *Nucleic Acids Res.*, 22: 1762-3, 1994).

A fusion gene between *mlt-13* and standard *gfp* was constructed using pPD95_81 as the PCR template. PCR reactions were performed under conditions described previously (Fraser et al., Nature 408:325-30, 2000). To generate the extrachromosomal arrays mgEx647, mgEx648, mgEx649, mgEx656, mgEx654, mgEx657, and mg659, the PCR products corresponding

to, respectively, *mlt-12::gfp-pest*, *mlt-13::gfp-pest*, *mlt-18::gfp-pest*, *mlt-24::gfp-pest*, *acn-1::gfp-pest*, *mlt-10::gfp-pest*, and *mlt-13::gfp*, each at 10 ng/ul , were microinjected into temperature-sensitive *pha-1(e2123)* mutant animals along with the *pha-1(+)* plasmid pBX (6) at 3ng/ul and pBS DNA at 87 ng/ul, allowing for the recovery and cultivation of worm populations in which virtually all animals maintained the fusion genes, because only *pha-1(+)* transgenic embryos survive at 25°C (Kamath et al., Nature 421:231-7, 2003). To verify that GFP-PEST molecules are degraded by the proteosome, we found that RNAi of the proteosome subunit gene *pbs-5* sustained fluorescence from *mlt-10::gfp* in larvae arrested for 2 days.

Use of the *pha-1 (e2123)* genetic background allowed for the cultivation of worm populations in which virtually all animals expressed the extrachromosal array, because only transgenic animals expressing *pha-1*(+) survive embryonic development at 25°C (Granato et al., *Nucleic Acids Res.*, 22: 1762-3, 1994). Temporal oscillations in gene expression were observed as changes in GFP-fluorescence over the period of a single molting cycle. Worms were visualized by Nomarski optics using standard techniques, and fluorescence was quantified using OPENLAB software (Improvision Inc. Lexington, MA).

Monitoring *mlt::gfp* fusion gene expression

To monitor temporal expression of the *mlt* gene *gfp* fusion genes, synchronized L1 hatchlings of GR1348, GR1349, GR1350, or GR1351 (Table 5) were plated on NGM with *E. coli* strain OP50 as a food source and incubated at 25°C. Fluorescent larvae were selected 14 hours later to ensure the use of non-mosaic, highly synchronous animals. Larvae were scored once every hour for detectable fluorescence, using a Zeiss Stemi-SV6 microscope, and for molting, indicated by shedding of the cuticle. Each animal was transferred to a new plate after each molt. In Figure 2, we report the percentage of animals that were fluorescent over time, on a scale normalized to the period

between molts for each worm under observation. As an example, a larvae that molted from L1 to L2 at noon, molted from L2 to L3 at 8 PM, was fluorescent at 7 p.m. and 8 p.m., and was not fluorescent at 6 p.m. or 9 p.m. would be recorded as fluorescent from time 1.75 to time 2.0, or, from 87.5 to 100% of the L2 stage. Calculations of the average duration of fluorescence, with the 95% confidence interval, include observations from larvae during the L2, L3, and L4 stages. Because many of the extrachromosomal arrays were associated with some larval lethality, only larvae that completed all four molts were included in the final analysis. A total of 24 larvae were analyzyed for *mlt-12::gfp*; 20, for the other reporters.

Fluorescence from all six *gfp* fusion genes was observed in epithelial cells that secrete cuticle, in larvae and, in some cases, late embryos. All six reporters were expressed in the hypodermis and, for *mlt-13*, *mlt-18*, *mlt-24*, and *acn-1*, also in the lateral seam cells, which are essential for molting and morphogenesis of the cuticle. Figures 2A-2D show that a pulse of fluorescence was observed in the hypodermis prior to each of the four molts, for all six *gfp* fusion genes. Fluorescence from *mlt-12::gfp* was first detected approximately 3 hours before the L1/L2 molt, which occurred roughly 17 hours after starved hatchlings were fed and cultivated at 25°C. The intensity of fluorescence increased until lethargus, a brief period when larvae cease moving or feeding before molting, and then decreased rapidly, such that fluorescence was barely detectable 2 hours after the molt (Figure 2A). Monitoring individual *Ex*[*mlt-12::gfp*] larvae over the course of development, fluorescence was observed starting at 65 ± 2 % and ending at 90 ± 2 % of the way through each larval stage (Figure 2B).

Cultivation of worms at 15°C delayed the first appearance of fluorescence in L1 larvae, and the first molt, by approximately 15 hours, and also expanded the period between peaks in fluorescence and between molts to the same extent. Similarly, the pulse of hypodermal expression for the *mlt-13* or *mlt-10* reporters began, respectively, 64 ± 3% or 63 ± 2% of the way through

each larval stage. Hypodermal fluorescence from *mlt-18::gfp* was detected earlier, from 51 ± 2% to 72 ± 3% of each stage, suggesting that MLT-18 antiprotease synthesized midway through a larval stage might repress proteases that are post-translationally activated at ecdysis. Fluorescence from *mlt-13::gfp* and *mlt-18::gfp* in seam cells also cycled in phase with molting, but often preceded and persisted longer than fluorescence in the hypodermis (Figure 2C).

Figures 3A-3H show that fluorescence associated with *Pmlt18::GFP-PEST* was detectable in the hypodermis during late intermolt and intensified until ecydsis. After ecydsis, fluorescence dissipated rapidly and did not increase until the onset of the next molt. Fluorescence associated with *Pmlt-13::GFP-PEST* was observed in the anterior cells of the hypodermis during lethargus and molting, and in the seam cells when they underwent division, close to the time of lethargus (Figure 3G and 3H). Fluorescence associated with *Pmlt-12::GFP-PEST* was observed in the hypodermis shortly before each of the four molts. The ability of the *mlt-12, mlt-13, and mlt-18* promoters to drive cyclic GFP expression in synchrony with the molting cycle identifies these genes as components of a periodic gene expression program required for molting. Moreover, the expression, timing, and pattern of *mlt-12* in hypodermis and of *mlt-13* and *mlt-18* in both hypodermis and seam cells is consistent with a role for these genes in ecdysis, given that hypodermal cells secrete cuticle and seam cells are required for molting.

Northern Analysis

To verify that cycling fluorescence from a *gfp-pest* fusion gene reflects dynamic temporal regulation of gene expression, we examined the level of one *mlt* gene message by northern analysis. The abundance of *mlt-10* mRNA in late L4 larvae exceeded that of mid L4 larvae by a factor of 6, and *mlt-10*

mRNA was barely detectable in young adults (Figure 2D), consistent with the observation that fluorescence from *mlt-10::gfppest* peaks late in each larval stage.

For northern analysis, RNA from extracts of mid L4, late L4, and young adult animals was resolved and hybridized with a *mlt-10* probe, corresponding to base pair 5070 to 6997 of cosmid C09E8 (GenBank Accession No:AF077529) (Lee et al., Science 300:644-647, 2003). Message levels were quantified using Imagequant software and a phosphorimager.

To order gene expression cascades, synchronized hatchlings of GR1348 and GR1349 were fed bacteria expressing dsRNA for each gene of interest, or, as a comtrol, fed isogenic bacteria not expressing dsRNA for a worm gene. After incubation for no more than 15 hours at 25°C, single, fluorescent larvae were transferred to 24 well RNAi plates seeded with the appropriate bacteria. For each developmental stage, larvae were observed over a 6 to 9 hour time period starting when control larvae first became fluorescent, and scored every 2 to 3 hours for detectable fluorescence and for the Mlt phenotype. In Figures 4A and 4B, we report the percentage of animals that were fluorescent prior to a defective molt, normalized to the fraction of control larvae that were fluorescent before molting from the same stage. Note that RNAi of *mlt-12* or *acn-1* prevented completion of the L2/L3 molt, whereas RNAi of *qhg-1*, *mlt-16*, or *mlt-13* interfered most often with the L3/L4 or L4/A molts. RNAi of *nhr-23* prevented completion of the L2/L3 molt in most *Ex*[*mlt-12::gfp*] larvae, but interfered with the L3/L4 or L4/A molts in *Ex*[*mlt-10::gfp*] larvae. Fluorescence was observed in 95% (n=56), 100% (n=43), or 94% (n=48) of control *Ex*[*mlt-10::gfp*] larvae during, respectively, the L2, L3, or L4 stage. Fluorescence was observed in 74% (n=57) or 70% (n=36) of L2 stage *Ex*[*mlt-12::gfp*] larvae, and in 90% (n=49) of L4 stage *Ex*[*mlt-12::gfp*] larvae.

To screen the full set of molting gene inactivations, approximately 20 synchronized hatchlings of GR1348 were fed each bacterial clone in 24 well format, in two trials. The percent of larvae with detectable fluorescence was scored 1 to 3 hours before the L2/L3, L3/L4, and L4/A molts, when the majority of control GR1348 larvae were fluorescent.

Fluorescence from particular *gfp* fusion genes was also observed in specialized epithelia including the rectal gland, rectal epithelia, the excretory duct and pore cells, and vulval precursors (Figure 5). Interestingly, the *acn-1* fusion gene was also expressed in the excretory gland cell of larvae (Figure 5). This gland cell may release or receive endocrine signals regulating molting (Chitwood, *Crit Rev Biochm Mol Biol* 34:273-84, 1999), and ACN-1 produced in the gland could regulate such an endocrine output. RNAi of *acn-1* likely reduces expression in the gland cell, since RNAi of gfp reduces fluorescence from *acn-1::gfp* in the entire excretory system. Fluorescence from *mlt-12::GFP* was also observed in a single posterior neuron that remains to be identified.

Taken together, the spatio-temporal expression pattern of *gfp* fusion genes suggests that *mlt-10*, *mlt-12*, *mlt-13*, *mlt-24*, *mlt-18*, and *acn-1* are expressed transiently before molting in epithelial cells that synthesize cuticle, and thus define a periodic gene expression program essential for molting. The upstream regulators driving *mlt* gene expression might also control collagen and nuclear hormone receptor genes whose expression oscillates over the molting cycle (Johnstone et al., *EMBO J* 15:3633-9, 1996).

Newly-identified *mlt* genes may be organized into genetic pathways using epistasis analysis. One strategy for organizing the newly-identified *mlt* genes into genetic pathways is to examine the expression of the *Pmlt-GFP-PEST* reporter genes in larvae undergoing RNAi against each of the newly-identified *mlt* genes.

The nuclear hormone receptor gene, *nhr-23,* was inactivated by RNAi (as described above) in *Ex[Pmlt-12::GFP-PEST]* larvae. GFP fluorescence was then detected by fluorescence microscopy at the time of the L3/L4 or L4/adult molt. Fluorescence associated with *Pmlt-12::GFP-PEST* was often not detectable in Mlt nematodes newly trapped in cuticle. In contrast, fluorescence associated with *Pmlt-12::GFP-PEST* was detected in Mlt nematodes undergoing RNAi against *lrp-1*, *rme-8*, *mlt-24*, or *mlt-26*. Control larvae, which were Non-Mlt larvae fed bacteria transformed with an empty vector, also displayed *Pmlt-12::GFP-PEST* fluorescence.

This observation, that *nhr-23(RNAi)* larvae carrying *mlt-12::gfp* or *mlt-10::gfp* failed to become fluorescent prior to their unsuccessful molt (Figure 4A), suggested that the nuclear hormone receptor NHR-23, synthesized in epithelial cells (Kostrouchova Dev. 125:1617-26, 1998), initiates the pulse of *mlt* gene expression late in each larval stage, thereby provoking an epithelial response to an endocrine cue for molting. Consistently, inactivation of *nhr-23* diminished hypodermal fluorescence from *mlt-24::gfp* and *mlt-18::gfp*. Signaling via NHR-23 may coordinate collagen production with the synthesis of MLT proteins that direct cuticle assembly, since *nhr-23* also drives expression of the cuticle collagen gene *dpy-7* (Kostrouchova et al., *PNAS* 98:7360-5, 2001). Moreover, MLT-12 likely functions downstream of NHR-23 in a regulatory cascade, since inactivation of *mlt-12* also abrogates expression of *mlt- 10::gfp*, but not of *mlt-12::gfp* (Figure 3A). MLT-12 secreted from the hypodermis could serve as an autocrine signal for molting, but could also signal to muscle cells, or providefeedback to neurons.

The majority of *acn-1(RNAi)* larvae also failed to express either *mlt-12::gfp* or *mlt-10::gfp* before an unsuccessful molt (Figure 4A), consistent with the view that ACN-1 synthesized in the hypodermis or excretory gland functions in the endocrine phase of molting. In contrast, after inactivation of the hedgehog-like gene *qhg-1*, the *fibrillin* homolog *mlt-16,* or the novel gene *mlt-13*, as many larvae expressed the fusion genes as did control larvae molting

from the same developmental stage, suggesting that these genes function downstream of, or in parallel to, induction of *mlt-10*, in the execution phase of molting.

To order the action of additional molting genes, we monitored fluorescence from *mlt-10::gfp* in 58 gene inactivations. Populations of *Ex*[*mlt-10::gfp*] larvae fed each dsRNA were observed late in the L2, L3, and L4 stages. Inactivation of five genes abrogated expression of *mlt-10::gfp* in 85% or more of larvae during one stage, and blocked development shortly thereafter (Figure 4B). The five genes, Y41D4B.10, W09B6.1, D1054.15, M03F8.3, and Y48B6A.3, likely function upstream of *mlt-10,* and encode, respectively, a secretory protein resembling delta/serrate ligands, acetyl-Coenzyme A carboxylase, homologs of the RNA splicing factors PLRG-1 (Ajuh et al., JBC 276:42370-81, 2001), or CRN (Chung et al. *RNA* 5:1042-54, 1999; Chung et al., *Biochim Biophys Acta* 1576: 287-97, 2002), and an exoribonuclease 54% identical to human XRN2 (Zhang et al., *Genomics* 59:252-4, 1999). Since microRNAs regulate developmental transitions in *C. elegans* (Reinhart et al., *Nature* 403:901-6, 2000), one intriguing possibility is that the product of Y48B6A.3 negatively regulates the abundance of one or more microRNAs whose target genes drive the L4-to-Adult molt. Among *Ex*[*mlt-10::gfp*] larvae fed 34 other dsRNAs, an equal or greater fraction became fluorescent ascontrol larvae of the same stage (Figure 4B). Molting-defective, fluorescent larvae were observed upon inactivation of *mlt-24*, F45G2.5, ZK430.8, *unc-52*, W10G6.3, *kin-2*, *bli-1*, and *DuOx,* strongly suggesting that the genes function downstream, or in parallel, to *mlt-10* expression.

By analogy with arthropods, we expect that neuroendocrine cues initiate molting in *C. elegans*, ultimately stimulating epithelial cells to synthesize a new cuticle and release the old one. Together, gene annotations, expression patterns, and ordering experiments suggested that our screen identified several

endocrine regulators of molting, including MLT-12, ACN-1, and NHR-23, as well as enzymes and ECM components essential for remodeling the exoskeleton.

Similar epistatic analyses are expected to place many, if not all, of the new *mlt* genes into genetic pathways characterized by early steps associated with neuroendocrine signaling or later steps promoting escape from the old cuticle.

Ecdysozoan Orthologs

DNA sequences corresponding to *mlt* genes of interest were retrieved from the repositories of sequence information at either the NCBI website (http://www.ncbi.nlm.nih.gov/) or wormbase (www.wormbase.org). The DNA sequence was then used for standard translating blast [tBLASTN] searching using the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). The DNA sequence corresponding to the top ortholog candidate produced by tblastn was retrieved from Genbank (http://www.ncbi.nlm.nih.gov/) and used for a BLASTx search of *C. elegans* proteins using the wormbase site (http://www.wormbase.org/db/searches/blast). These methods provide for the identification of orthologs of *C. elegans mlt* genes (Tables 1A, 1B, 4A-4D, and 7) revealed by our RNAi analysis. An ortholog is a protein that is highly related to a reference sequence. One skilled in the art would expect an ortholog to functionally substitute for the reference sequence. Tables 4A-4D and 7 list exemplary orthologs by Genbank accession number.

C. elegans gene: M6.1

Species	EST ID	Assession Number	E value
Ascaris suum	ki02g09.y1	gb|BM280603	1e-28
Ascaris suum	kk52b05.y1	gb|BQ382546	1e-26
Ascaris suum	As_L3_09B01_SKPL	gb|BI594018	1e-25
Ascaris suum	kj92f03.y1	gb|BM965152	3e-24
Ascaris suum	As_nc_10C07_SKPL	gb|BI594311	1e-22
Ascaris suum	ki08f11.y1	gb|BM281039	2e-18
Brugia malayi	SWYD25CAU14E02SK	gb|AW675831	8e-19
Brugia malayi	SWYACAL08E03SK	gb|BE758356	5e-18
Haemonchus contortus	Hc_d11_11E10_SKPL	gb|BF060126	4e-25
Haemonchus contortus	Hc_d11_18E03_SKPL	gb|BF422872	2e-20
Haemonchus contortus	Hc_d11_09G03_SKPL	gb|BF059991	1e-16
Meloidogyne incognita	rd19e10.y1	gb|BQ613722	8e-25
Meloidogyne incognita	rd02c03.y1	gb|BQ613170	1e-24
Meloidogyne incognita	rd08a12.y1	gb|BQ613497	2e-24
Meloidogyne hapla	rf48d08.y1	gb|BQ836630	1e-21
Onchocerca volvulus	SWOv3MCAM52D01SK	gb|BF824665	4e-16
Onchocerca volvulus	SWOvL3CAN13E07	gb|AA917260	2e-19
Ostertagia ostertagi	ph69a09.y1	gb|BQ099825	7e-20
Strongyloides ratti	kt51c06.y4	gb|BI742464	8e-19
Strongyloides stercoralis	kq58d04.y1	gb|BF014961	3e-28
Strongyloides stercoralis	kq25d02.y1	gb|BE579290	7e-20
Strongyloides stercoralis	kq31d11.y1	gb|BE579614	1e-20
Strongyloides stercoralis	kq07e05.y1	gb|BG227475	1e-19
Strongyloides stercoralis	kq38a11.y1	gb|BE580177	3e-19

Toxocara canis	ko17e01.y1	gb|BM965806	4e-16
Trichinella spiralis	ps41c08.y1	gb|BG353660	5e-26
Trichinella spiralis	ps21c11.y4	gb|BG732010	3e-20
Trichuris muris	Tm_ad_32C10_SKPL	gb|BM174670	8e-32

C. elegans gene: ZC101.2

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000659084026	gb|BM601480	6e-31
Anopheles gambiae	17000687479592	gb|BM596670	3e-20
Anopheles gambiae	17000687506857	gb|BM598004	2e-15
Anopheles gambiae	17000687368906	gb|BM588620	4e-15
Anopheles gambiae	17000687134459	gb|BM612519	5e-15
Anopheles gambiae	17000687565373	gb|BM637990	2e-13
Aedes aegypti	AEMTBL28	gb|AI618963	5e-23
Ancylostoma caninum	pb38e07.y1	gb|BQ666249	5e-13
Ascaris suum	kh43d01.y1	gb|BI782862	1e-13
Bombyx mori	AV399222	dbj|AV399222	2e-21
Brugia malayi	BSBmL3SZ44P24SK	gb|AI723671	5e-60
Brugia malayi	SWL4CAK11D03SK	gb|AW600207	9e-53
Brugia malayi	SWYD25CAU01B01SK	gb|AW179566	2e-45
Brugia malayi	MB3D6V3B03T3	gb|AA661133	2e-27
Brugia malayi	SWYD25CAU13H12SK	gb|AW676004	4e-25
Dirofilaria immitis	ke10h02.y1	gb|BQ454813	5e-58
Dirofilaria immitis	ke15g10.y1	gb|BQ454884	2e-14
Globodera rostochiensis	GE1828	gb|AW506417	1e-14
Haemonchus contortus	Hc_d11_08E04_SKPL	gb|BE496755	3e-99
Ancylostoma caninum	pa32g09.y1	gb|BE352403	4e-19
Meloidogyne hapla	rc29b02.y1	gb|BM901402	2e-44
Meloidogyne hapla	rc45d03.y1	gb|BM901130	3e-40
Meloidogyne hapla	rc47g03.y1	gb|BM901696	3e-39
Meloidogyne incognita	rd08a06.y1	gb|BQ613494	3e-21

Meloidogyne incognita	MD0294	gb|BE217664	2e-15
Onchocerca volvulus	SWOvL2CAS04B06SK	gb|AW980135	4e-62
Onchocerca volvulus	SWOvL3CAN52A02SK	gb|AI132759	7e-43
Onchocerca volvulus	SWOv3MCAM25F01SK	gb|AI581466	4e-14
Onchocerca volvulus	SWOvL3CAN18G05	gb|AI096109	4e-44
Onchocerca volvulus	SWOv3MCA770SK	gb|AA294548	7e-36
Parastrongyloides trichosuri	kx99e03.y2	gb|BM513799	1e-14
Strongyloides ratti	kt72h10.y1	gb|BI323571	3e-44
Strongyloides ratti	kt75c08.y3	gb|BI502464	2e-39
Strongyloides ratti	kt20f09.y1	gb|BG894201	1e-36
Strongyloides ratti	kt33h03.y1	gb|BI073703	2e-21
Strongyloides ratti	kt27e05.y3	gb|BI450558	9e-17
Strongyloides stercoralis	kq04h11.y1	gb|BG227295	1e-49
Strongyloides stercoralis	kq42h08.y1	gb|BE581152	1e-49
Strongyloides stercoralis	kq26e04.y1	gb|BE579360	2e-32
Toxocara canis	ko14a04.y1	gb|BM965583	9e-59

C. elegans gene: D1054.15

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687163725	gb|BM577379	3e-71
Anopheles gambiae	17000687054314	gb|BM600555	1e-37
Anopheles gambiae	17000687477449	gb|BM595864	6e-25
Amblyomma variegatum	EST575203	gb|BM292661	6e-33
Ancylostoma caninum	pa80h05.y1	gb|BG232752	4e-77
Meloidogyne arenaria	rm16b05.y1	gb|BI863068	2e-87
Globodera pallida	OP20173	gb|BM415102	4e-56
Necator americanus	Na_L3_47E12_SAC	gb|BU088714	e-108
Onchocerca volvulus	SWOvMfCAR07F05SK	gb|BE202350	9e-49
Pristionchus pacificus	rs62h03.y1	gb|AW115214	3e-29
Trichinella spiralis	ps30a03.y2	gb|BG520170	2e-28

C. elegans gene: Y37D8A.10

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687279294	gb|BM583815	2e-24
Anopheles gambiae	17000687137751	gb|BM612986	1e-22
Anopheles gambiae	17000687067307	gb|BM601081	8e-22
Bombyx mori	AV402441	dbj|AV402441	4e-28
Haemonchus contortus	Hc_L3_04D09_SKPL	gb|BI595303	3e-68
Heterodera glycines	ro61h02.y3	gb|BI396794	1e-29
Heterodera glycines	ro73d01.y1	gb|BI749054	4e-27
Meloidogyne arenaria	rm39d08.y1	gb|BI747379	4e-30
Meloidogyne arenaria	rm23g05.y1	gb|BI746177	3e-26
Meloidogyne incognita	rb29e05.y1	gb|BM882772	4e-24
Meloidogyne incognita	ra93b11.y1	gb|BM774415	2e-20
Necator americanus	Na_L3_32G04_SAC	gb|BU666155	9e-27
Necator americanus	Na_L3_27B01_SAC	gb|BU088007	3e-26
Parastrongyloides trichosuri	kx55a12.y1	gb|BI744051	3e-29
Pristionchus pacificus	rs33b11.y1	gb|AW052236	7e-51
Strongyloides stercoralis	kp31d05.y1	gb|BE029374	1e-37
Ancylostoma ceylanicum	pj18d12.y1	gb|BQ288481	2e-59
Ancylostoma ceylanicum	pj18b06.y1	gb|BQ288451	4e-57
Ancylostoma ceylanicum	pj18f04.y1	gb|BQ288495	4e-57
Ancylostoma ceylanicum	pj19a07.y1	gb|BQ288871	4e-57
Ancylostoma ceylanicum	pj19e09.y1	gb|BQ288915	4e-57
Ancylostoma ceylanicum	pj19f07.y1	gb|BQ288924	4e-57
Ancylostoma ceylanicum	pj19g09.y1	gb|BQ288934	4e-57
Ancylostoma ceylanicum	pj20b03.y1	gb|BQ289634	4e-57

Ancylostoma ceylanicum	pj21b01.y1	gb|BQ289718	4e-57
Ancylostoma ceylanicum	pj21d08.y1	gb|BQ289743	4e-57
Ancylostoma ceylanicum	pj21e04.y1	gb|BQ289749	4e-57
Ancylostoma ceylanicum	pj22a07.y1	gb|BQ289455	4e-57
Ancylostoma ceylanicum	pj22b06.y1	gb|BQ289464	4e-57
Ancylostoma ceylanicum	pj22h05.y1	gb|BQ289530	4e-57
Ancylostoma ceylanicum	pj23a11.y1	gb|BQ289548	4e-57
Ancylostoma ceylanicum	pj23d03.y1	gb|BQ289565	4e-57
Ancylostoma ceylanicum	pj24d12.y1	gb|BQ289067	4e-57
Ancylostoma ceylanicum	pj24e03.y1	gb|BQ289070	2e-57
Ancylostoma ceylanicum	pj24g03.y1	gb|BQ289088	2e-57
Ancylostoma ceylanicum	pj25b04.y1	gb|BQ288958	4e-57
Ancylostoma ceylanicum	pj25c06.y1	gb|BQ288971	4e-57
Ancylostoma ceylanicum	pj26c09.y1	gb|BQ289134	3e-57
Ancylostoma ceylanicum	pj28d09.y1	gb|BQ289322	4e-57
Ancylostoma ceylanicum	pj28e11.y1	gb|BQ289334	4e-57
Ancylostoma ceylanicum	pj28f04.y1	gb|BQ289338	2e-57
Ancylostoma ceylanicum	pj28f07.y1	gb|BQ289341	2e-57
Ancylostoma ceylanicum	pj28h06.y1	gb|BQ289361	2e-57
Ancylostoma ceylanicum	pj29c04.y1	gb|BQ289391	4e-57
Ancylostoma ceylanicum	pj29d03.y1	gb|BQ289401	4e-57
Ancylostoma ceylanicum	pj30e06.y1	gb|BQ288830	4e-57
Ancylostoma ceylanicum	pj30g06.y1	gb|BQ288847	4e-57
Ancylostoma ceylanicum	pj30h03.y1	gb|BQ288855	4e-57
Ancylostoma ceylanicum	pj31a06.y1	gb|BQ288703	4e-57
Ancylostoma ceylanicum	pj31c11.y1	gb|BQ288727	2e-57

Ancylostoma ceylanicum	pj31d01.y1	gb|BQ288729	2e-57
Ancylostoma ceylanicum	pj31d04.y1	gb|BQ288732	4e-57
Ancylostoma ceylanicum	pj33d04.y1	gb|BQ288645	2e-57
Ancylostoma ceylanicum	pj33d09.y1	gb|BQ288650	4e-57
Ancylostoma ceylanicum	pj33g10.y1	gb|BQ288684	4e-57
Ancylostoma ceylanicum	pj33h04.y1	gb|BQ288689	4e-57
Ancylostoma ceylanicum	pj34a03.y1	gb|BQ274663	2e-57
Ancylostoma ceylanicum	pj34d06.y1	gb|BQ274700	4e-57
Ancylostoma ceylanicum	pj35e01.y1	gb|BQ274789	2e-57
Ancylostoma ceylanicum	pj35e12.y1	gb|BQ274800	4e-57
Ancylostoma ceylanicum	pj35f05.y1	gb|BQ274803	2e-57
Ancylostoma ceylanicum	pj36c11.y1	gb|BQ275536	4e-57
Ancylostoma ceylanicum	pj36e10.y1	gb|BQ275566	4e-57
Ancylostoma ceylanicum	pj38a12.y1	gb|BQ274837	4e-57
Ancylostoma ceylanicum	pj38b02.y1	gb|BQ274838	4e-57
Ancylostoma ceylanicum	pj38g07.y1	gb|BQ274896	4e-57
Ancylostoma ceylanicum	pj38g12.y1	gb|BQ274900	4e-57
Ancylostoma ceylanicum	pj39f02.y1	gb|BQ274962	4e-57
Ancylostoma ceylanicum	pj39g08.y1	gb|BQ274977	4e-57
Ancylostoma ceylanicum	pj39h11.y1	gb|BQ274990	4e-57
Ancylostoma ceylanicum	pj40b05.y1	gb|BQ275007	4e-57
Ancylostoma ceylanicum	pj40b06.y1	gb|BQ275008	4e-57
Ancylostoma ceylanicum	pj40b11.y1	gb|BQ275012	4e-57
Ancylostoma ceylanicum	pj41e03.y1	gb|BQ275122	2e-57
Ancylostoma ceylanicum	pj41e07.y1	gb|BQ275126	4e-57
Ancylostoma ceylanicum	pj41f02.y1	gb|BQ275133	4e-57

Ancylostoma ceylanicum	pj42b02.y1	gb|BQ275176	4e-57
Ancylostoma ceylanicum	pj42b12.y1	gb|BQ275185	4e-57
Ancylostoma ceylanicum	pj42c11.y1	gb|BQ275195	4e-57
Ancylostoma ceylanicum	pj42e03.y1	gb|BQ275208	4e-57
Ancylostoma ceylanicum	pj42g08.y1	gb|BQ275233	2e-57
Ancylostoma ceylanicum	pj43a09.y1	gb|BQ275256	4e-57
Ancylostoma ceylanicum	pj43b04.y1	gb|BQ275262	4e-57
Ancylostoma ceylanicum	pj43d07.y1	gb|BQ275287	4e-57
Ancylostoma ceylanicum	pj43e04.y1	gb|BQ275295	4e-57
Ancylostoma ceylanicum	pj45c09.y1	gb|BQ275446	4e-57
Ancylostoma ceylanicum	pj45c12.y1	gb|BQ275449	4e-57
Ancylostoma ceylanicum	pj46f09.y1	gb|BQ275735	4e-57
Ancylostoma ceylanicum	pj46f12.y1	gb|BQ275738	4e-57
Ancylostoma ceylanicum	pj47g06.y1	gb|BQ275825	5e-57
Ancylostoma ceylanicum	pj48a03.y1	gb|BQ275842	4e-57
Ancylostoma ceylanicum	pj48a11.y1	gb|BQ275850	4e-57
Ancylostoma ceylanicum	pj48b09.y1	gb|BQ275860	4e-57
Ancylostoma ceylanicum	pj48e12.y1	gb|BQ275895	4e-57
Ancylostoma ceylanicum	pj50g03.y1	gb|BQ276059	2e-57
Ancylostoma ceylanicum	pj50g07.y1	gb|BQ276063	4e-57
Ancylostoma ceylanicum	pj51b04.y1	gb|BQ276091	4e-57
Ancylostoma ceylanicum	pj51g04.y1	gb|BQ276145	2e-57
Ancylostoma ceylanicum	pj53c01.y1	gb|BQ288078	4e-57
Ancylostoma ceylanicum	pj54d07.y1	gb|BQ288160	4e-57
Ancylostoma ceylanicum	pj56c04.y1	gb|BQ288297	4e-57
Ancylostoma ceylanicum	pj56f08.y1	gb|BQ288327	4e-57

Ancylostoma ceylanicum	pj57c06.y1	gb|BQ288376	2e-57
Ancylostoma ceylanicum	pj57g03.y1	gb|BQ288415	4e-57
Ancylostoma ceylanicum	pj19c08.y1	gb|BQ288893	7e-56
Ancylostoma ceylanicum	pj19g02.y1	gb|BQ288927	4e-56
Ancylostoma ceylanicum	pj20f10.y1	gb|BQ289683	4e-56
Ancylostoma ceylanicum	pj21f06.y1	gb|BQ289758	2e-56
Ancylostoma ceylanicum	pj22b10.y1	gb|BQ289468	4e-56
Ancylostoma ceylanicum	pj23e11.y1	gb|BQ289589	1e-56
Ancylostoma ceylanicum	pj24g11.y1	gb|BQ289093	5e-56
Ancylostoma ceylanicum	pj24h06.y1	gb|BQ289100	1e-56
Ancylostoma ceylanicum	pj25c04.y1	gb|BQ288969	2e-56
Ancylostoma ceylanicum	pj26a09.y1	gb|BQ289111	1e-56
Ancylostoma ceylanicum	pj27d08.y1	gb|BQ289233	2e-56
Ancylostoma ceylanicum	pj27f06.y1	gb|BQ289254	9e-56
Ancylostoma ceylanicum	pj28b02.y1	gb|BQ289292	5e-56
Ancylostoma ceylanicum	pj28e06.y1	gb|BQ289330	9e-56
Ancylostoma ceylanicum	pj28h04.y1	gb|BQ289359	5e-56
Ancylostoma ceylanicum	pj30f04.y1	gb|BQ288837	2e-56
Ancylostoma ceylanicum	pj31a12.y1	gb|BQ288708	4e-56
Ancylostoma ceylanicum	pj31h01.y1	gb|BQ288774	4e-56
Ancylostoma ceylanicum	pj32a08.y1	gb|BQ288531	2e-56
Ancylostoma ceylanicum	pj32g07.y1	gb|BQ288598	2e-56
Ancylostoma ceylanicum	pj33a10.y1	gb|BQ288621	4e-56
Ancylostoma ceylanicum	pj34g06.y1	gb|BQ274732	2e-56
Ancylostoma ceylanicum	pj34g09.y1	gb|BQ274734	1e-56
Ancylostoma ceylanicum	pj35d10.y1	gb|BQ274788	9e-56

Ancylostoma ceylanicum	pj35e05.y1	gb|BQ274793	2e-56
Ancylostoma ceylanicum	pj35g08.y1	gb|BQ274814	2e-56
Ancylostoma ceylanicum	pj37e02.y1	gb|BQ275637	2e-56
Ancylostoma ceylanicum	pj38d11.y1	gb|BQ274868	7e-56
Ancylostoma ceylanicum	pj38e07.y1	gb|BQ274876	9e-56
Ancylostoma ceylanicum	pj38f07.y1	gb|BQ274885	9e-56
Ancylostoma ceylanicum	pj39e10.y1	gb|BQ274958	9e-56
Ancylostoma ceylanicum	pj39f12.y1	gb|BQ274970	2e-56
Ancylostoma ceylanicum	pj40e07.y1	gb|BQ275043	2e-56
Ancylostoma ceylanicum	pj40f11.y1	gb|BQ275056	4e-56
Ancylostoma ceylanicum	pj40h07.y1	gb|BQ275074	7e-56
Ancylostoma ceylanicum	pj41e06.y1	gb|BQ275125	2e-56
Ancylostoma ceylanicum	pj43a07.y1	gb|BQ275254	2e-56
Ancylostoma ceylanicum	pj44d05.y1	gb|BQ275371	7e-56
Ancylostoma ceylanicum	pj46b08.y1	gb|BQ275695	2e-56
Ancylostoma ceylanicum	pj46h04.y1	gb|BQ275751	4e-56
Ancylostoma ceylanicum	pj47e05.y1	gb|BQ275803	5e-56
Ancylostoma ceylanicum	pj47g03.y1	gb|BQ275822	9e-56
Ancylostoma ceylanicum	pj48e07.y1	gb|BQ275890	9e-56
Ancylostoma ceylanicum	pj48h05.y1	gb|BQ275920	2e-56
Ancylostoma ceylanicum	pj51c10.y1	gb|BQ276107	1e-56
Ancylostoma ceylanicum	pj51d07.y1	gb|BQ276116	2e-56
Ancylostoma ceylanicum	pj51f12.y1	gb|BQ276141	4e-56
Ancylostoma ceylanicum	pj53b08.y1	gb|BQ288073	4e-56
Ancylostoma ceylanicum	pj53b11.y1	gb|BQ288076	4e-56
Ancylostoma ceylanicum	pj53d09.y1	gb|BQ288094	9e-56

Ancylostoma ceylanicum	pj55d09.y1	gb|BQ288231	1e-56
Ancylostoma ceylanicum	pj56a11.y1	gb|BQ288274	3e-56
Ancylostoma ceylanicum	pj47g05.y1	gb|BQ275824	2e-55
Ancylostoma ceylanicum	pj49g12.y1	gb|BQ275986	8e-55
Ancylostoma ceylanicum	pj50e07.y1	gb|BQ276042	1e-55
Ancylostoma ceylanicum	pj21d03.y1	gb|BQ289738	3e-53
Ancylostoma ceylanicum	pj24c02.y1	gb|BQ289052	3e-53
Ancylostoma ceylanicum	pj23h05.y1	gb|BQ289614	2e-52
Ancylostoma ceylanicum	pj28a01.y1	gb|BQ289280	2e-52
Ancylostoma ceylanicum	pj36d11.y1	gb|BQ275548	2e-52
Ancylostoma ceylanicum	pj38f01.y1	gb|BQ274881	3e-51
Ancylostoma ceylanicum	pj50f12.y1	gb|BQ276056	1e-50
Ancylostoma ceylanicum	pj43h10.y1	gb|BQ275332	2e-48
Ancylostoma ceylanicum	pj20g08.y1	gb|BQ289691	8e-44
Ancylostoma ceylanicum	pj45g02.y1	gb|BQ275483	2e-24

C. elegans gene: W01F3.3

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687309881	gb|BM642414	9e-24
Ancylostoma caninum	pb20b05.y1	gb|BM077795	2e-19
Haemonchus contortus	Hc_d11_18C12_SKPL	gb|BF422862	9e-18
Caenorhabditis briggsae	pk19f02.r1	gb|R04105	2e-33
Meloidogyne arenaria	rm30c06.y1	gb|BI746672	6e-31
Meloidogyne incognita	rb02g12.y1	gb|BM882536	6e-30
Meloidogyne incognita	rd16d07.y1	gb|BQ625515	3e-25
Meloidogyne incognita	ra89g12.y1	gb|BM774133	1e-10
Meloidogyne hapla	rc37e10.y1	gb|BM902581	5e-19
Meloidogyne hapla	rc54a09.y2	gb|BQ089876	4e-08
Meloidogyne javanica	rk82f08.y3	gb|BI745590	9e-18
Necator americanus	Na_L3_31A05_SA	gb|BU666009	1e-21
Strongyloides ratti	kt12a05.y2	gb|BG893620	2e-19
Strongyloides ratti	kt12a06.y2	gb|BG893621	2e-19
Strongyloides ratti	kt36a12.y1	gb|BI073867	2e-19
Strongyloides ratti	kt32b02.y1	gb|BI073544	4e-19
Strongyloides ratti	kt15d05.y1	gb|BG893793	5e-18
Strongyloides stercoralis	kq39d09.y1	gb|BE580288	3e-20
Strongyloides stercoralis	kq19h11.y1	gb|BG226301	9e-18
Trichuris muris	Tm_ad_30H04_SKPL	gb|BM174557	9e-21
Trichinella spiralis	pt03g01.y1	gb|BQ692168	2e-08

C. elegans gene: T24H7.2

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687490394	gb|BM597171	8e-26
Bombyx mori	AU003373	dbj|AU003373	8e-34
Bombyx mori	AU000515	dbj|AU000515	4e-33
Bombyx mori	AU000521	dbj|AU000521	1e-33
Bombyx mori	AU003962	dbj|AU003962	9e-32
Bombyx mori	AV405756	dbj|AV405756	5e-30
Bombyx mori	AU003842	dbj|AU003842	6e-29
Bombyx mori	AU003119	dbj|AU003119	2e-28
Bombyx mori	AU002974	dbj|AU002974	9e-27
Bombyx mori	AU004644	dbj|AU004644	9e-27
Bombyx mori	AV406070	dbj|AV406070	1e-27
Bombyx mori	AV401482	dbj|AV401482	2e-26
Bombyx mori	AV398101	dbj|AV398101	2e-25
Bombyx mori	AU003732	dbj|AU003732	6e-25
Bombyx mori	AU004041	dbj|AU004041	2e-25
Bombyx mori	AU005109	dbj|AU005109	1e-25
Bombyx mori	AU000006	dbj|AU000006	7e-24
Bombyx mori	AU004834	dbj|AU004834	2e-24
Bombyx mori	AU002644	dbj|AU002644	2e-23
Bombyx mori	AU002841	dbj|AU002841	2e-23
Bombyx mori	AU004017	dbj|AU004017	2e-23
Bombyx mori	AU004636	dbj|AU004636	1e-23
Bombyx mori	AV404009	dbj|AV404009	1e-23
Helicoverpa armigera	DH03D07	gb|BU038682	1e-28

Helicoverpa armigera	DH03C12	gb|BU038678	3e-26
Meloidogyne incognita	rd23c04.y1	gb|BQ548270	1e-30
Parastrongyloides trichosuri	kx97e04.y2	gb|BM513653	1e-40
Parastrongyloides trichosuri	kx91g06.y1	gb|BM513534	4e-40
Parastrongyloides trichosuri	kx97e04.y1	gb|BM514195	4e-40
Parastrongyloides trichosuri	kx91h03.y1	gb|BM513542	2e-39
Parastrongyloides trichosuri	kx94f07.y1	gb|BM514994	1e-37
Parastrongyloides trichosuri	kx88e07.y1	gb|BM513356	6e-37
Parastrongyloides trichosuri	kx94a01.y1	gb|BM514944	2e-28
Strongyloides stercoralis	kp73b04.y1	gb|BE223128	3e-32

C. elegans gene: C23F12.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687479257	gb|BM596487	4e-28
Anopheles gambiae	17000687145608	gb|BM614899	1e-13
Anopheles gambiae	17000687373180	gb|BM651037	2e-12
Anopheles gambiae	17000687310164	gb|BM586066	3e-10
Meloidogyne hapla	rc36h09.y1	gb|BM902526	2e-24
Meloidogyne incognita	MD0572	gb|BE238916	6e-17
Meloidogyne hapla	rc35f02.y1	gb|BM902409	1e-16
Onchocerca volvulus	SWOvAFCAP28B08SK	gb|AI539970	2e-38
Strongyloides stercoralis	kq38g03.y1	gb|BE580231	8e-35
Strongyloides stercoralis	kq18a07.y1	gb|BG226155	4e-32

C. elegans gene: M03F4.7

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687321631	gb|BM587777	6e-30
Anopheles gambiae	17000687087727	gb|BM609137	1e-28
Ancylostoma caninum	pb41c07.y1	gb|BQ666411	e-111
Ancylostoma caninum	pb46d07.y1	gb|BQ666710	e-110
Ancylostoma caninum	pb55f03.y1	gb|BQ667584	e-108
Ancylostoma caninum	pb44g02.y1	gb|BQ666619	e-107
Ancylostoma caninum	pb56d04.y1	gb|BQ667626	e-107
Ancylostoma caninum	pb40e01.y1	gb|BQ666362	e-104
Ancylostoma caninum	pb41f01.y1	gb|BQ666431	e-104
Ancylostoma caninum	pb07g02.y1	gb|BI744344	2e-99
Ancylostoma caninum	pb61h06.y1	gb|BQ667467	3e-92
Ancylostoma caninum	pb24a04.y1	gb|BM129955	7e-87
Ancylostoma caninum	pb34e05.y1	gb|BQ125307	3e-83
Ancylostoma caninum	pb27c03.y1	gb|BM130151	5e-80
Ancylostoma caninum	pb36b05.y1	gb|BQ666117	3e-70
Anopheles gambiae	4A3B-AAC-F-10-F	emb|AJ283528	6e-37
Ascaris suum	ki65c01.y1	gb|BM319475	e-103
Ascaris suum	ki31h12.y1	gb|BM284247	3e-99
Ascaris suum	ki05h11.y1	gb|BM280852	8e-93
Ascaris suum	kk76f12.y1	gb|BU966016	1e-92
Ascaris suum	kk07a07.y1	gb|BQ095577	2e-80
Ascaris suum	ki29d01.y1	gb|BM284064	8e-67
Brugia malayi	kb09b01.y1	gb|BM889340	1e-78
Brugia malayi	kb21h09.y1	gb|BU781519	4e-78

Brugia malayi	kb05d10.y1	gb|BM889092	2e-77
Brugia malayi	kb08c11.y1	gb|BM889289	4e-76
Brugia malayi	kb09a09.y1	gb|BM889336	4e-75
Brugia malayi	kb35a07.y1	gb|BU917823	1e-72
Brugia malayi	kb33g08.y1	gb|BU917746	4e-43
Brugia malayi	SWYD25CAU08A09SK	gb|AW257642	2e-33
Haemonchus contortus	Hc_d11_05C04_SAC	gb|BF059828	8e-56
Haemonchus contortus	pw13a11.y1	gb|CA033609	1e-62
Meloidogyne hapla	rc40d09.y1	gb|BM900690	2e-83
Meloidogyne hapla	rc29a06.y1	gb|BM901456	1e-74
Globodera pallida	OP20499	gb|BM415425	1e-66
Onchocerca volvulus	SWOvAMCAQ03E09SK	gb|AI095964	6e-40
Ostertagia ostertagi	ph54b09.y1	gb|BM896658	3e-79
Ostertagia ostertagi	ph54b06.y1	gb|BM896656	4e-77
Ostertagia ostertagi	ph50g05.y1	gb|BM896993	1e-66
Pristionchus pacificus	rs17c06.y1	gb|AI986802	2e-52
Pristionchus pacificus	rs36d12.y1	gb|AW052520	3e-48
Strongyloides ratti	kt77b06.y2	gb|BI450741	2e-98
Strongyloides ratti	kt25b12.y3	gb|BI450405	8e-83
Strongyloides ratti	kt77b06.y1	gb|BI142485	1e-76
Strongyloides stercoralis	kp90f12.y1	gb|BG226555	1e-79
Strongyloides stercoralis	kp93c08.y1	gb|BG226767	4e-85
Strongyloides stercoralis	kq44d02.y1	gb|BE581256	5e-85
Strongyloides stercoralis	kq32e11.y1	gb|BE579808	3e-58
Toxocara canis	ko07h06.y1	gb|BM966480	9e-90
Toxocara canis	ko09d02.y1	gb|BM966578	8e-90

Toxocara canis	ko29c01.y1	gb|BQ089597	7e-81

C. elegans gene: K04F10.4

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687506656	gb|BM633120	1e-24
Anopheles gambiae	17000687507484	gb|BM633599	6e-23
Ancylostoma caninum	pb41a04.y1	gb|BQ666394	2e-24
Ancylostoma caninum	pb45d03.y1	gb|BQ666654	2e-24
Ancylostoma caninum	pb55h11.y1	gb|BQ667604	2e-24
Ancylostoma caninum	pb57d02.y1	gb|BQ667675	4e-22
Ancylostoma caninum	pb56c12.y1	gb|BQ667624	2e-21
Ancylostoma caninum	pb06d11.y1	gb|BI744250	4e-20
Ancylostoma caninum	pb06e07.y1	gb|BI744258	5e-20
Ancylostoma caninum	pb62e01.y1	gb|BQ667504	1e-17
Ancylostoma caninum	pb51a04.y1	gb|BQ667006	2e-15
Apis mellifera	BB170002B20B06.5	gb|BI503119	5e-27
Globodera rostochiensis	GE2051	gb|AW506559	8e-34
Haemonchus contortus	pw14h05.y1	gb|CA033722	1e-95
Meloidogyne hapla	rc48c03.y1	gb|BM901742	2e-20
Meloidogyne hapla	rf27a01.y1	gb|BQ837484	1e-20
Meloidogyne hapla	rc47e08.y1	gb|BM901678	2e-19
Meloidogyne hapla	rf69b12.y1	gb|BU094482	7e-14
Meloidogyne incognita	rb16a10.y1	gb|BM880593	9e-14
Meloidogyne incognita	ra87a11.y1	gb|BM773890	1e-13
Necator americanus	Na_L3_17G04_SAC	gb|BU087198	4e-14
Ostertagia ostertagi	ph25b11.y2	gb|BQ099039	5e-18
Ostertagia ostertagi	ph25d06.y2	gb|BQ099057	3e-13
Parastrongyloides trichosuri	kx11d08.y3	gb|BI451155	2e-63

Parastrongyloides trichosuri	kx09d05.y3	gb|BI322885	2e-58
Parastrongyloides trichosuri	kx14f11.y3	gb|BI322659	9e-54
Parastrongyloides trichosuri	kx13e05.y3	gb|BI322554	8e-50
Parastrongyloides trichosuri	kx37f06.y1	gb|BI743006	3e-37
Parastrongyloides trichosuri	kx35g09.y1	gb|BI742844	4e-35
Parastrongyloides trichosuri	kx38c05.y1	gb|BI743068	2e-12
Strongyloides ratti	ku14a12.y1	gb|BQ091197	2e-18
Strongyloides stercoralis	kp21e05.y1	gb|BE028912	7e-24
Strongyloides stercoralis	kp31f09.y1	gb|BE029399	4e-24
Strongyloides stercoralis	kp25f12.y1	gb|BE029166	2e-22
Strongyloides stercoralis	kp72e12.y1	gb|BG225849	1e-19
Strongyloides stercoralis	kp41h12.y1	gb|BE030358	7e-16
Strongyloides stercoralis	kp70g06.y1	gb|BG225690	7e-16
Strongyloides stercoralis	kp68c10.y1	gb|BG225473	3e-15
Strongyloides stercoralis	kp74h04.y1	gb|BE223285	2e-14
Strongyloides stercoralis	kp40c03.y1	gb|BE030223	1e-13
Strongyloides stercoralis	kp40g11.y1	gb|BE030270	2e-12
Strongyloides stercoralis	kq43e03.y1	gb|BE581195	2e-73
Strongyloides stercoralis	kq11c12.y1	gb|BG227598	5e-42
Strongyloides stercoralis	kp96f07.y1	gb|BG227075	2e-41
Strongyloides stercoralis	kq35e07.y1	gb|BE579996	3e-18
Trichinella spiralis	pt11b03.y1	gb|BQ693113	1e-51
Trichinella spiralis	pt15a05.y1	gb|BQ692444	7e-27
Trichinella spiralis	ps89g02.y1	gb|BQ541838	4e-19
Trichinella spiralis	pt08f06.y1	gb|BQ692908	3e-18
Trichinella spiralis	pt10c09.y1	gb|BQ693042	5e-18

Trichinella spiralis	pt02e07.y1	gb|BQ692074	1e-15

C. elegans gene: F41C3.4

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687149117	gb|BM616703	2e-27
Anopheles gambiae	17000687069029	gb|BM602087	3e-22
Anopheles gambiae	17000687370128	gb|BM649918	5e-16
Anopheles gambiae	17000687307553	gb|BM585633	9e-16
Anopheles gambiae	AL692646	emb|AL692646	7e-11
Ancylostoma caninum	pj14f02.y1	gb|BM131161	3e-52
Brugia malayi	MBAFCX8E03T3	gb|AA509202	2e-11
Haemonchus contortus	pw09h01.y1	gb|CA034321	8e-46
Haemonchus contortus	pw04h10.y1	gb|CA033875	2e-45
Haemonchus contortus	pw06g03.y1	gb|CA034012	2e-45
Haemonchus contortus	pw11c02.y1	gb|CA033489	2e-45
Haemonchus contortus	pw11f07.y1	gb|CA033516	2e-45
Haemonchus contortus	pw13f10.y1	gb|CA033653	2e-45
Haemonchus contortus	pw16e06.y1	gb|CA033344	2e-45
Haemonchus contortus	pw07e03.y1	gb|CA034184	3e-44
Haemonchus contortus	pw11b07.y1	gb|CA033483	3e-43
Haemonchus contortus	pw14c04.y1	gb|CA033687	9e-37
Haemonchus contortus	pw11a08.y1	gb|CA033477	1e-22
Meloidogyne arenaria	rm17b07.y1	gb|BI745692	1e-32
Strongyloides ratti	kt15c03.y1	gb|BG893781	8e-20

C. elegans gene: F49C12.12

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687157397	gb|BM617424	5e-13
Anopheles gambiae	17000659084146	gb|BM603802	7e-13
Anopheles gambiae	17000687163115	gb|BM576950	7e-13
Anopheles gambiae	17000687275479	gb|BM582159	7e-13
Anopheles gambiae	17000687478936	gb|BM623580	7e-13
Anopheles gambiae	17000687493042	gb|BM625373	7e-13
Ancylostoma caninum	pb02e11.y1	gb|BF250630	1e-22
Ancylostoma caninum	pa80g12.y1	gb|BG232750	1e-13
Ancylostoma ceylanicum	pj34c09.y1	gb|BQ274691	1e-34
Ancylostoma ceylanicum	pj26b10.y1	gb|BQ289124	1e-33
Ancylostoma ceylanicum	pj47a06.y1	gb|BQ275763	4e-33
Ancylostoma ceylanicum	pj53e04.y1	gb|BQ288100	6e-33
Ancylostoma ceylanicum	pj55c11.y1	gb|BQ288222	2e-33
Bombyx mori	AU004305	dbj|AU004305	9e-13
Bombyx mori	AV404505	dbj|AV404505	1e-12
Globodera rostochiensis	GE1768	gb|AW506351	2e-36
Heterodera glycines	ro14f12.y1	gb|BF013515	2e-36
Manduca sexta	EST1141	gb|BF047044	6e-12
Meloidogyne hapla	rf52c12.y2	gb|BU094732	2e-30
Meloidogyne javanica	rk98d03.y1	gb|BI745272	3e-12
Necator americanus	Na_L3_52B05_SAC	gb|BU089096	9e-37
Necator americanus	Na_L3_13A10_SAC	gb|BU086831	1e-36
Ostertagia ostertagi	ph82h03.y1	gb|BQ457787	1e-05
Parastrongyloides trichosuri	kx83e06.y1	gb|BM513019	5e-30

Parastrongyloides trichosuri	kx83a12.y1	gb|BM512987	9e-19
Strongyloides stercoralis	kp36g11.y1	gb|BE029934	1e-15
Trichinella spiralis	ps85c06.y1	gb|BQ543136	4e-17
Trichinella spiralis	ps01f05.y1	gb|BG232803	2e-14
Trichuris muris	Tm_ad_31C04_SKPL	gb|BM174586	2e-19

C. elegans gene: C01H6.5

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687115955	gb|BM611525	1e-18
Anopheles gambiae	17000687438370	gb|BM618330	2e-18
Ascaris suum	ki20a12.y1	gb|BM281749	2e-39
Ascaris suum	ki04c07.y1	gb|BM280724	3e-21
Ascaris suum	kj40b03.y1	gb|BM568658	3e-21
Apis mellifera	BB160005B10B06.5	gb|BI511357	1e-50
Apis mellifera	BB160003A10G01.5	gb|BI510638	2e-23
Apis mellifera	BB160016A20C12.5	gb|BI514819	1e-22
Apis mellifera	BB160017A10C06.5	gb|BI514984	2e-18
Bombyx mori	AU000440	dbj|AU000440	6e-27
Bombyx mori	AV398791	dbj|AV398791	7e-19
Trichinella spiralis	ps26g10.y1	gb|BG353339	3e-29

C. elegans gene: F57B9.2

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687476900	gb|BM622947	7e-41
Anopheles gambiae	4A3A-AAY-A-12-R	emb|AJ282447	2e-25
Ancylostoma caninum	pj60d02.y3	gb|BU780997	6e-53
Amblyomma variegatum	EST577652	gb|BM291118	2e-51
Meloidogyne incognita	rb13e12.y1	gb|BM881751	3e-36

C. elegans gene: C09G5.6

Species	EST ID	Assession Number	E value
Ascaris suum	MBAsBWA298M13R	gb|AW165858	1e-26
Ascaris suum	ki01e01.y1	gb|BM280488	3e-26
Ascaris lumbricoides	Al_am_43C11_T3	gb|BU586933	6e-25
Ascaris suum	MBAsBWA064M13R	gb|AW165746	6e-25
Ascaris suum	MBAsBWA101M13R	gb|AW165662	6e-25
Ascaris suum	As_bw_11D06_M13R	gb|BG733657	7e-25
Ascaris suum	kh96f09.y1	gb|BM285267	5e-24
Ascaris suum	kh93f02.y1	gb|BM285005	3e-23
Ascaris suum	kh94c02.y1	gb|BM285056	3e-22
Ascaris suum	kh98c07.y1	gb|BM284719	2e-21
Ascaris suum	As_bw_11D11_M13R	gb|BG733660	1e-21
Ascaris suum	MBAsBWA069M13R	gb|AW165751	6e-20
Ascaris suum	MBAsBWA079M13R	gb|AW165757	1e-20
Ascaris suum	ki03c09.y1	gb|BM280644	1e-20
Ascaris suum	ki10h03.y1	gb|BM281210	1e-20
Ascaris lumbricoides	Al_am_36G05_T3	gb|BU586727	4e-19
Ascaris suum	MBAsBWA115M13R	gb|AW165673	3e-19
Ascaris lumbricoides	Al_am_06E07_T3	gb|BU585487	2e-18
Ascaris suum	MBAsBWA108M13R	gb|AW165669	9e-18
Ascaris suum	ki07g08.y1	gb|BM280986	9e-18
Ascaris suum	As_nc_11A05_SKPL	gb|BI594341	1e-17
Brugia malayi	SWBmL3SDI01B01SK	gb|AI066836	3e-22
Brugia malayi	SWBmL3SBH08A07SK	gb|AA933446	5e-21
Brugia malayi	SWYD25CAU13E10SK	gb|AW675970	1e-21

Brugia malayi	SWYD25CAU07H07SK	gb|AW225415	3e-18
Brugia malayi	SWYD25CAU08E01SK	gb|AW257678	4e-18
Brugia malayi	SWAMCAC16G06SK	gb|AI083297	2e-18
Brugia malayi	MBAFCX3C05T3	gb|AA471504	3e-18
Globodera pallida	OP20201	gb|BM415129	1e-20
Onchocerca volvulus	SWOv3MCAM47A04SK	gb|BF482033	4e-19
Onchocerca volvulus	SWOv3MCAM54F12SK	gb|BF942751	1e-18
Onchocerca volvulus	SWOvAFCAP48F12SK	gb|BF114585	2e-18
Onchocerca volvulus	SWOv3MCAM54B04SK	gb|BF918253	5e-18
Onchocerca volvulus	SWOv3MCAM49B01SK	gb|BF599258	7e-18
Onchocerca volvulus	SWOv3MCAM56A04SK	gb|BG310491	7e-18
Onchocerca volvulus	SWOv3MCAM55E02SK	gb|BG310586	1e-17
Onchocerca volvulus	SWOv3MCAM58G11SK	gb|BF718930	2e-17
Onchocerca volvulus	SWOvL2CAS04B05SK	gb|AW980134	3e-18
Onchocerca volvulus	SWOvL2CAS12F11SK	gb|BE552486	2e-17
Onchocerca volvulus	SWOvL3CAN29D10SK	gb|AI511508	2e-17
Onchocerca volvulus	SWOv3MCA1795SK	gb|AA618829	4e-18
Onchocerca volvulus	SWOv3MCA1241SK	gb|AI111204	7e-18
Ostertagia ostertagi	Oo_L4_01H05_SKPL	gb|BG734092	1e-22
Ostertagia ostertagi	Oo_L4_02F08_SKPL	gb|BG734148	2e-20
Ostertagia ostertagi	Oo_L4_02F06_SKPL	gb|BG734146	1e-19
Ostertagia ostertagi	Oo_L4_02C04_SKPL	gb|BG734117	8e-19
Ostertagia ostertagi	Oo_L4_03D09_SKPL	gb|BG891779	1e-18
Strongyloides stercoralis	kq20e08.y1	gb|BG226349	7e-25
Strongyloides stercoralis	kq60b02.y1	gb|BF015009	7e-25
Strongyloides stercoralis	kp95e12.y1	gb|BG227018	1e-17

Strongyloides stercoralis	kq38g09.y1	gb|BE580236	1e-17

C. elegans gene: F38A1.8

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000668812767	gb|BM631762	3e-17
Anopheles gambiae	17000687134447	gb|BM612513	2e-14
Anopheles gambiae	17000687443762	gb|BM593736	1e-12
Anopheles gambiae	17000687151121	gb|BM617129	5e-11
Anopheles gambiae	17000687509165	gb|BM634491	4e-10
Ancylostoma caninum	pb34d04.y1	gb|BQ125296	6e-24
Anopheles gambiae	4A3A-AAY-A-03-F	emb|AJ280813	6e-29
Amblyomma variegatum	EST577711	gb|BM291177	6e-26
Amblyomma variegatum	EST575079	gb|BM292537	1e-21
Apis mellifera	BB160006B10D05.5	gb|BI511656	3e-25
Apis mellifera	BB160008B20D07.5	gb|BI512333	6e-15
Bombyx mori	AV400988	dbj|AV400988	1e-21
Meloidogyne javanica	rk65c03.y1	gb|BG736990	9e-17
Parastrongyloides trichosuri	kx31d03.y1	gb|BI501368	6e-31
Strongyloides stercoralis	kq13c10.y1	gb|BG227780	1e-24
Zeldia punctata	rp11b10.y1	gb|AW773519	4e-22

C. elegans gene: F54C9.2

Species	EST ID	Assession Number	E value
Amblyomma variegatum	EST577517	gb|BM290983	7e-35
Amblyomma variegatum	EST577724	gb|BM291190	2e-35
Bombyx mori	AU002973	dbj|AU002973	9e-39
Bombyx mori	AU003385	dbj|AU003385	7e-39
Bombyx mori	AV405994	dbj|AV405994	3e-39
Bombyx mori	AV401902	dbj|AV401902	7e-38
Bombyx mori	AV398157	dbj|AV398157	9e-38
Bombyx mori	AU000006	dbj|AU000006	2e-38
Bombyx mori	AV401963	dbj|AV401963	2e-37
Bombyx mori	AV402885	dbj|AV402885	7e-37
Bombyx mori	AU004017	dbj|AU004017	8e-37
Bombyx mori	AU006113	dbj|AU006113	4e-37
Bombyx mori	AU000664	dbj|AU000664	5e-36
Bombyx mori	AU003373	dbj|AU003373	1e-36
Bombyx mori	AU003442	dbj|AU003442	5e-36
Bombyx mori	AU003705	dbj|AU003705	2e-36
Bombyx mori	AU003286	dbj|AU003286	6e-35
Bombyx mori	AU004420	dbj|AU004420	6e-35
Bombyx mori	AU004716	dbj|AU004716	7e-35
Bombyx mori	AU006399	dbj|AU006399	5e-35
Bombyx mori	AV404137	dbj|AV404137	7e-35
Bombyx mori	AV405329	dbj|AV405329	3e-35
Bombyx mori	AV401750	dbj|AV401750	2e-34
Bombyx mori	AV398101	dbj|AV398101	5e-34

Bombyx mori	AU000646	dbj|AU000646	2e-34
Bombyx mori	AU003356	dbj|AU003356	2e-34
Bombyx mori	AU003364	dbj|AU003364	2e-34
Bombyx mori	AU003396	dbj|AU003396	3e-34
Bombyx mori	AU003686	dbj|AU003686	6e-34
Bombyx mori	AU003777	dbj|AU003777	1e-34
Bombyx mori	AU004205	dbj|AU004205	5e-34
Bombyx mori	AU004626	dbj|AU004626	2e-34
Bombyx mori	AU004827	dbj|AU004827	8e-34
Bombyx mori	AV404445	dbj|AV404445	3e-34
Bombyx mori	AV405771	dbj|AV405771	8e-34
Bombyx mori	AV405924	dbj|AV405924	2e-34
Bombyx mori	AV406118	dbj|AV406118	4e-34
Bombyx mori	AV398235	dbj|AV398235	2e-33
Bombyx mori	AV398367	dbj|AV398367	2e-33
Bombyx mori	AV398398	dbj|AV398398	2e-33
Bombyx mori	AU000243	dbj|AU000243	2e-33
Bombyx mori	AU002763	dbj|AU002763	2e-33
Bombyx mori	AU003119	dbj|AU003119	1e-33
Bombyx mori	AU003402	dbj|AU003402	2e-33
Bombyx mori	AU003811	dbj|AU003811	2e-33
Bombyx mori	AU004599	dbj|AU004599	2e-33
Bombyx mori	AU004708	dbj|AU004708	1e-33
Bombyx mori	AV404361	dbj|AV404361	2e-33
Bombyx mori	AV406241	dbj|AV406241	3e-33
Pristionchus pacificus	rs33h02.y1	gb|AW052295	1e-55

Strongyloides stercoralis	kq09h07.y1	gb|BG226148	1e-44

C. elegans gene: F08C6.1

Species	EST ID	Assession Number	E value
Strongyloides stercoralis	kq42f07.y1	gb|BE581131	2e-34

C. elegans gene: H04M03.4

Species	EST ID	Assession Number	E value
Brugia malayi	SWAMCAC31A02SK	gb|AI770981	2e-27
Brugia malayi	SWAMCA827SK	gb|AA007720	8e-21
Meloidogyne arenaria	rm17b03.y1	gb|BI745690	1e-61
Meloidogyne hapla	rc32c07.y1	gb|BM902109	2e-56
Meloidogyne hapla	rc62e03.y1	gb|BQ090180	9e-31
Meloidogyne hapla	rc51b07.y2	gb|BQ089651	9e-12
Onchocerca volvulus	SWOv3MCA840SK	gb|AA294602	5e-15
Onchocerca volvulus	SWOv3MCA233SK	gb|AA294264	2e-11
Strongyloides ratti	kt23d09.y3	gb|BI397280	1e-61
Strongyloides ratti	kt17d09.y1	gb|BG894044	1e-57
Strongyloides ratti	kt09f02.y1	gb|BG894269	3e-35
Strongyloides ratti	kt14e07.y1	gb|BG893462	3e-35
Strongyloides stercoralis	kq07b03.y1	gb|BG227443	2e-75
Strongyloides stercoralis	kq36f05.y1	gb|BE580066	1e-55

C. elegans gene: Y48B6A.3

Species	EST ID	Assession Number	E value
Ostertagia ostertagi	ph79d04.y1	gb|BQ457535	6e-52
Globodera rostochiensis	rr63d03.y1	gb|BM345416	3e-13
Strongyloides ratti	kt53e08.y3	gb|BI324097	6e-40

C. elegans gene: T27F2.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687243104	gb|BM580695	1e-37
Anopheles gambiae	17000687309019	gb|BM641931	1e-36
Apis mellifera	BB160009A10E09.5	gb|BI512416	2e-38
Meloidogyne hapla	rc43c01.y1	gb|BM900937	1e-33
Meloidogyne incognita	rb13h02.y1	gb|BM881774	8e-22
Necator americanus	Na_L3_16E01_SAC	gb|BU087096	1.4e-22

C. elegans gene: T14F9.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687110468	gb|BM610289	4e-75
Anopheles gambiae	17000687367798	gb|BM648864	1e-62
Anopheles gambiae	17000687565365	gb|BM637983	2e-58
Anopheles gambiae	17000687324034	gb|BM647561	2e-57
Anopheles gambiae	17000687560412	gb|BM635758	2e-56
Anopheles gambiae	17000687163827	gb|BM577458	2e-55
Anopheles gambiae	17000687119262	gb|BM611925	2e-54
Anopheles gambiae	17000668915702	gb|BM596992	8e-48
Anopheles gambiae	17000687377484	gb|BM653132	2e-45
Anopheles gambiae	17000687164768	gb|BM578365	1e-42
Anopheles gambiae	17000687499422	gb|BM629010	5e-41
Anopheles gambiae	17000687368814	gb|BM649439	2e-39
Anopheles gambiae	17000687496339	gb|BM597467	3e-38
Anopheles gambiae	17000687243041	gb|BM580645	6e-38
Anopheles gambiae	17000687384459	gb|BM590932	6e-38
Ancylostoma caninum	pb28e07.y1	gb|BM130242	4e-72
Ascaris suum	As_nc_16B02_SKPL	gb|BI594547	4e-67
Apis mellifera	BB170001B10D01.5	gb|BI504920	2e-39
Bombyx mori	AU003538	dbj|AU003538	3e-80
Bombyx mori	AU002118	dbj|AU002118	1e-44
Bombyx mori	AU006312	dbj|AU006312	5e-36
Globodera rostochiensis	rr09e06.y1	gb|BM345905	2e-73
Heterodera glycines	ro25h04.y1	gb|BF014612	2e-54
Heterodera glycines	ro28a10.y1	gb|BF014776	7e-54

Meloidogyne javanica	rk48d04.y1	gb|BG735889	5e-65
Globodera pallida	OP20152	gb|BM415082	2e-60
Necator americanus	Na_L3_10D12_SAC	gb|BU086612	5e-65
Parastrongyloides trichosuri	kx75e08.y1	gb|BM513291	5e-69
Strongyloides stercoralis	kq20f07.y1	gb|BG226359	5e-70
Strongyloides stercoralis	kq22d06.y1	gb|BE579107	2e-56

C. elegans gene: C34G6.6

Species	EST ID	Assession Number	E value
Ascaris suum	kh44c05.y1	gb|BI782938	9e-52
Brugia malayi	MBAFCX2B06T3	gb|AA471404	2e-68
Brugia malayi	SWAMCAC32C03SK	gb|AI795199	4e-63
Haemonchus contortus	Hc_d11_10F03_SKPL	gb|BF060055	4e-18
Meloidogyne javanica	rk89c03.y1	gb|BI744615	4e-44
Meloidogyne arenaria	rm18b11.y1	gb|BI745765	4e-10
Pristionchus pacificus	rs76h10.y1	gb|BI500192	2e-69
Strongyloides ratti	kt36b11.y1	gb|BI073876	1e-41
Strongyloides ratti	kt37a09.y1	gb|BI073944	2e-41
Strongyloides ratti	kt70c11.y1	gb|BI323373	1e-36
Strongyloides ratti	kt62e08.y1	gb|BI323179	2e-36
Strongyloides stercoralis	kq30c01.y1	gb|BE579677	2e-53
Strongyloides stercoralis	kq41b02.y1	gb|BE580410	1e-47
Strongyloides stercoralis	kq33h12.y1	gb|BE579888	4e-22
Strongyloides stercoralis	kq63d06.y1	gb|BF015363	7e-20
Strongyloides stercoralis	kq05d11.y1	gb|BG227329	3e-11
Trichuris muris	Tm_ad_12H10_SKPL	gb|BG577864	4e-12

C. elegans gene: T01H3.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687162874	gb|BM576761	2e-49
Anopheles gambiae	17000687307464	gb|BM585573	2e-49
Anopheles gambiae	17000659020522	gb|BM599204	1e-48
Anopheles gambiae	17000687372976	gb|BM589399	1e-48
Anopheles gambiae	17000687556220	gb|BM634907	1e-48
Anopheles gambiae	17000687310364	gb|BM642665	5e-48
Anopheles gambiae	17000687389290	gb|BM656815	5e-48
Anopheles gambiae	17000687496331	gb|BM597462	2e-47
Anopheles gambiae	17000687284475	gb|BM640730	2e-46
Anopheles gambiae	17000659202014	gb|BM618205	5e-45
Anopheles gambiae	17000687151325	gb|BM617284	3e-44
Anopheles gambiae	17000687308708	gb|BM641772	6e-44
Anopheles gambiae	17000687276191	gb|BM582703	3e-43
Anopheles gambiae	17000687042988	gb|BM599367	2e-42
Anopheles gambiae	17000687118079	gb|BM611782	2e-42
Anopheles gambiae	17000687108061	gb|BM609935	2e-40
Anopheles gambiae	17000687130255	gb|BM612274	4e-39
Anopheles gambiae	17000687322687	gb|BM646650	6e-39
Anopheles gambiae	17000687569900	gb|BM639302	4e-37
Anopheles gambiae	17000687383534	gb|BM654480	7e-37
Anopheles gambiae	17000687566347	gb|BM638292	7e-37
Anopheles gambiae	17000687145897	gb|BM615037	6e-36
Anopheles gambiae	17000687437980	gb|BM618257	2e-33
Anopheles gambiae	17000687498198	gb|BM628172	6e-30

Anopheles gambiae	17000687147324	gb|BM615629	3e-26
Amblyomma variegatum	EST574690	gb|BM292148	3e-49
Apis mellifera	BB170024A10E06.5	gb|BI509938	7e-48
Apis mellifera	BB160011B10C08.5	gb|BI513199	2e-33
Bombyx mori	AU005205	dbj|AU005205	2e-33
Globodera rostochiensis	GE1711	gb|AW506310	4e-67
Haemonchus contortus	Hc_ad_15H10_SKPL	gb|BM139010	2e-76
Ancylostoma caninum	pa18g12.y1	gb|AW627173	8e-25
Heterodera glycines	ro10e01.y1	gb|BF013645	4e-46
Heterodera glycines	ro84c04.y1	gb|BI748962	1e-20
Zeldia punctata	rp06a10.y1	gb|AW773378	3e-60
Zeldia punctata	rp01e11.y1	gb|AW783702	3e-52
Manduca sexta	EST968	gb|BF046871	2e-35
Meloidogyne javanica	rk60d11.y1	gb|BG736647	3e-39
Meloidogyne javanica	rk74g05.y1	gb|BI142836	1e-39
Necator americanus	Na_L3_37A02_SAC	gb|BU666330	8e-33
Strongyloides ratti	kt49e06.y4	gb|BI502419	6e-38
Strongyloides stercoralis	kq25f06.y1	gb|BE579307	3e-29
Trichinella spiralis	ps31h12.y2	gb|BG438616	1e-50
Trichinella spiralis	pt25f08.y1	gb|BQ737954	1e-50

C. elegans gene: F38H4.9

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687077251	gb|BM605757	e-110
Anopheles gambiae	17000687112957	gb|BM610732	2e-99
Anopheles gambiae	17000687498699	gb|BM628538	3e-98
Anopheles gambiae	17000687491478	gb|BM624539	1e-95
Anopheles gambiae	17000687162264	gb|BM576307	2e-93
Anopheles gambiae	17000687237697	gb|BM579205	9e-91
Anopheles gambiae	17000687494575	gb|BM626183	1e-90
Anopheles gambiae	17000687373656	gb|BM651182	3e-87
Anopheles gambiae	17000687387542	gb|BM656160	3e-87
Anopheles gambiae	17000687439479	gb|BM618770	3e-87
Anopheles gambiae	17000687138537	gb|BM613259	9e-83
Anopheles gambiae	17000687386006	gb|BM591368	2e-77
Anopheles gambiae	17000687075820	gb|BM605128	3e-77
Anopheles gambiae	17000687444639	gb|BM594603	2e-75
Anopheles gambiae	17000687311718	gb|BM643158	9e-75
Ascaris suum	kh42g04.y1	gb|BI782814	8e-89
Ascaris suum	ki30c03.y1	gb|BM284127	9e-80
Ascaris suum	kj60c12.y1	gb|BM569375	3e-55
Amblyomma variegatum	EST576450	gb|BM289916	2e-74
Apis mellifera	BB170030B20B04.5	gb|BI507201	7e-80
Bombyx mori	AU000600	dbj|AU000600	3e-91
Bombyx mori	AU000644	dbj|AU000644	3e-91
Meloidogyne javanica	rk93b04.y1	gb|BI744849	4e-79
Necator americanus	Na_L3_35H12_SAC	gb|BU666328	e-118

Necator americanus	Na_L3_16C05_SAC	gb|BU087079	1e-99
Necator americanus	Na_L3_17H12_SAC	gb|BU087214	1e-37
Necator americanus	Na_L3_51B04_SAC	gb|BU089013	5e-20
Ostertagia ostertagi	ph05a12.y2	gb|BQ097609	e-104
Ostertagia ostertagi	ph08g10.y2	gb|BQ097814	2e-99
Parastrongyloides trichosuri	kx48h12.y1	gb|BI863834	2e-69

C. elegans gene: K09H9.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687438560	gb|BM592590	1e-26
Anopheles gambiae	17000687439428	gb|BM618729	1e-25
Brugia malayi	SWAMCAC19C09SK	gb|AI083314	8e-27
Sarcoptes scabiei	ESSU0232	gb|BG817810	4e-27
Strongyloides stercoralis	kp98c02.y1	gb|BG227182	2e-28
Trichinella spiralis	ps51c09.y1	gb|BG520770	4e-21

C. elegans gene: F54A5.1

Species	EST ID	Assession Number	E value
Parastrongyloides trichosuri	kx21a02.y1	gb|BI451197	4e-40

C. elegans gene: F33A8.1

Species	EST ID	Assession Number	E value
Bombyx mori	AV405747	dbj|AV405747	6e-58
Meloidogyne incognita	MD0049	gb|BE191668	3e-36

C. elegans gene: ZK686.3

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687101940	gb|BM609414	1e-46
Anopheles gambiae	17000687160079	gb|BM617864	3e-44
Anopheles gambiae	17000687499984	gb|BM629333	7e-40
Anopheles gambiae	17000687320084	gb|BM586921	6e-39
Anopheles gambiae	17000687564993	gb|BM637706	2e-38
Anopheles gambiae	17000687385741	gb|BM655170	1e-37
Anopheles gambiae	17000687441179	gb|BM593025	1e-35
Anopheles gambiae	17000687087920	gb|BM609287	2e-32
Anopheles gambiae	17000687113255	gb|BM610801	1e-29
Anopheles gambiae	17000668938573	gb|BM636391	9e-21
Anopheles gambiae	4A3A-AAO-F-10-R	emb|AJ282089	5e-25
Anopheles gambiae	4A3A-ABC-G-08-R	emb|AJ282843	2e-19
Apis mellifera	EST242	gb|BE844497	1e-25
Apis mellifera	EST241	gb|BE844496	2e-10
Amblyomma variegatum	EST575352	gb|BM292810	7e-52
Amblyomma variegatum	EST574536	gb|BM291994	3e-51
Apis mellifera	BB160010B10H06.5	gb|BI512874	1e-34
Bombyx mori	AU004344	dbj|AU004344	1e-52
Brugia malayi	MBAFCW6H10T3	gb|AA842318	8e-22
Brugia malayi	SWMFCA462SK	gb|AA022342	1e-21
Globodera pallida	pal201	gb|AW505639	1e-32
Haemonchus contortus	Hc_d11_21A04_SKPL	gb|BF423018	9e-74
Haemonchus contortus	Hc_d11_13F09_SKPL	gb|BF060296	4e-36
Caenorhabditis briggsae	pk41g11.s1	gb|R05170	8e-33

Necator americanus	Na_L3_04C08_SAC	gb|BG467473	6e-22
Pristionchus pacificus	rs40g12.y1	gb|AW097184	9e-71
Pristionchus pacificus	rs30c07.y1	gb|AI989236	4e-29
Strongyloides stercoralis	kq49c03.y1	gb|BE581316	2e-48
Strongyloides stercoralis	kq08h12.y1	gb|BG226083	3e-47
Strongyloides stercoralis	kq23b07.y1	gb|BE579155	4e-27

C. elegans gene: F09B12.1

Species	EST ID	Assession Number	E value
Onchocerca volvulus	SWOv3MCAM23F06SK	gb|AI665735	4e-10
Strongyloides stercoralis	kq19a02.y1	gb|BG226227	1e-23
Strongyloides stercoralis	kq43f01.y1	gb|BE581202	1e-13
Trichuris muris	Tm_ad_03C11_SKPL	gb|BF169279	5e-11

C. elegans gene: K07D8.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687507565	gb|BM633656	3e-15
Brugia malayi	BSBmL3SZ15A23SK	gb|AI783143	1e-66
Meloidogyne incognita	MD0517	gb|BE238861	8e-38
Strongyloides ratti	kt27g02.y3	gb|BI450575	3e-38
Strongyloides ratti	kt88d03.y1	gb|BI502339	6e-33
Strongyloides stercoralis	kp75c05.y1	gb|BE223322	2e-23

C. elegans gene: ZK1073.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687445431	gb|BM620760	3e-29
Anopheles gambiae	17000687311462	gb|BM642979	3e-27
Anopheles gambiae	17000687069233	gb|BM602173	8e-27
Anopheles gambiae	17000687085881	gb|BM608010	8e-17
Anopheles gambiae	17000687277442	gb|BM583063	2e-15
Anopheles gambiae	17000668639510	gb|BM629367	1e-13
Anopheles gambiae	17000687379911	gb|BM654068	2e-13
Amblyomma variegatum	EST577485	gb|BM290951	9e-23
Amblyomma variegatum	EST575426	gb|BM292884	8e-19
Amblyomma variegatum	EST576458	gb|BM289924	2e-19
Amblyomma variegatum	EST574248	gb|BM291706	2e-18
Amblyomma variegatum	EST574565	gb|BM292023	2e-18
Amblyomma variegatum	EST575109	gb|BM292567	2e-18
Amblyomma variegatum	EST575360	gb|BM292818	1e-18
Amblyomma variegatum	EST575673	gb|BM293144	2e-18
Amblyomma variegatum	EST576512	gb|BM289978	5e-18
Amblyomma variegatum	EST576929	gb|BM290395	4e-18
Amblyomma variegatum	EST577334	gb|BM290800	2e-18
Amblyomma variegatum	EST576568	gb|BM290034	2e-17
Amblyomma variegatum	EST576853	gb|BM290319	9e-15
Bombyx mori	AV400999	dbj|AV400999	1e-20
Bombyx mori	AV400998	dbj|AV400998	4e-15
Globodera rostochiensis	rr26f04.y1	gb|BM355559	1e-50
Globodera rostochiensis	rr08g01.y1	gb|BM345835	3e-35

Ancylostoma caninum	pa49f11.y1	gb|AW735249	6e-46
Heterodera glycines	ro77a08.y1	gb|BI749346	4e-37
Heterodera glycines	ro60f02.y3	gb|BI396703	4e-26
Heterodera glycines	ro76c03.y1	gb|BI749286	2e-25
Heterodera glycines	ro57a04.y4	gb|BI451623	6e-16
Heterodera glycines	ro75g12.y1	gb|BI749253	1e-16
Meloidogyne incognita	rd12e01.y1	gb|BQ548499	7e-73
Meloidogyne arenaria	rm15c02.y1	gb|BI863000	3e-15
Ostertagia ostertagi	ph39b03.y1	gb|BM897271	9e-34
Parastrongyloides trichosuri	kx43h07.y1	gb|BI743414	1e-31
Pristionchus pacificus	rs88f09.y1	gb|BM320361	9e-92
Pristionchus pacificus	rt04c04.y2	gb|BM566361	1e-23
Strongyloides ratti	kt66c09.y1	gb|BI323694	2e-57
Strongyloides stercoralis	kp87b07.y1	gb|BE223687	1e-36
Strongyloides stercoralis	kq04g11.y1	gb|BG227286	8e-59
Trichinella spiralis	pt34f08.y1	gb|BQ693400	3e-52
Trichinella spiralis	pt41e05.y1	gb|BQ739201	4e-42
Trichinella spiralis	ps06g08.y1	gb|BG302307	3e-34

C. elegans gene: CD4.4

Species	EST ID	Assession Number	E value
Bombyx mori	AU003753	dbj|AU003753	2e-06
Pratylenchus penetrans	pz11e06.y1	gb|BQ626542	2e-19
Pratylenchus penetrans	pz21d10.y1	gb|BQ580851	2e-19
Pratylenchus penetrans	pz28a06.y1	gb|BQ626857	1e-06
Pristionchus pacificus	rs39f03.y1	gb|AW097092	8e-24
Pristionchus pacificus	rs53g02.y1	gb|AW114710	5e-18
Pristionchus pacificus	rs37f03.y1	gb|AW052618	2e-14
Trichinella spiralis	ps05d02.y2	gb|BG519941	6e-11
Trichinella spiralis	ps05d02.y3	gb|BG521059	3e-11

C. elegans gene: F11C1.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687115955	gb|BM611525	3e-41
Ascaris suum	ki04c07.y1	gb|BM280724	6e-20
Ascaris suum	kj40b03.y1	gb|BM568658	6e-20
Ascaris suum	ki20a12.y1	gb|BM281749	5e-18
Ascaris suum	kk53a06.y1	gb|BQ382607	4e-18
Ascaris suum	kh20b07.y1	gb|BI783431	1e-17
Ascaris suum	kk28e12.y1	gb|BQ381181	1e-17
Ascaris suum	kk34c05.y1	gb|BQ381563	1e-17
Ascaris suum	kk36a10.y1	gb|BQ382856	1e-17
Ascaris suum	kk40g10.y1	gb|BQ383122	1e-17
Ascaris suum	kk58c01.y1	gb|BQ383209	1e-17
Apis mellifera	BB160003A10G01.5	gb|BI510638	4e-22
Apis mellifera	BB160005B10B06.5	gb|BI511357	7e-19
Apis mellifera	BB160017A10C06.5	gb|BI514984	4e-18
Apis mellifera	BB160016A20C12.5	gb|BI514819	1e-17
Bombyx mori	AU000440	dbj|AU000440	3e-17
Globodera rostochiensis	rr19d05.y1	gb|BM354985	2e-17
Strongyloides stercoralis	kq42c09.y1	gb|BE581104	1e-27

C. elegans gene: F16B4.3

Species	EST ID	Assession Number	E value
Pristionchus pacificus	rs06b03.r1	gb|AA191781	2e-13

C. elegans gene: Y38F2AL.3

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687310422	gb|BM642705	2e-46
Anopheles gambiae	17000687111489	gb|BM610534	1e-41
Anopheles gambiae	17000687374739	gb|BM651831	2e-36
Anopheles gambiae	17000687444802	gb|BM594743	2e-35
Anopheles gambiae	17000687564429	gb|BM637448	1e-29
Bombyx mori	AU005959	dbj|AU005959	6e-64
Brugia malayi	BSBmMFSZ08G14SK	gb|AI007333	2e-79
Globodera rostochiensis	rr24f03.y1	gb|BM355406	1e-69
Caenorhabditis briggsae	pk05f06.s1	gb|R03292	2e-32
Meloidogyne arenaria	rm27a09.y1	gb|BI746435	2e-80
Parastrongyloides trichosuri	kx20h12.y3	gb|BI322419	3e-44
Pristionchus pacificus	rs80f06.y1	gb|BI500714	1e-82
Strongyloides ratti	kt66a07.y1	gb|BI323674	5e-47
Strongyloides ratti	kt46f02.y3	gb|BI323910	7e-39
Strongyloides stercoralis	kq10b05.y1	gb|BG227519	1e-69
Strongyloides stercoralis	kq50d07.y1	gb|BE581674	8e-64
Strongyloides stercoralis	kq41h12.y1	gb|BE580542	4e-42
Strongyloides stercoralis	kp45f12.y1	gb|BG224376	2e-29
Trichinella spiralis	pt40c10.y1	gb|BQ739097	2e-65
Trichinella spiralis	ps03d04.y3	gb|BG520983	1e-49
Trichinella spiralis	pt07a03.y1	gb|BQ692776	2e-45
Trichinella spiralis	ps03d04.y1	gb|BG302151	5e-37
Trichinella spiralis	ps12g06.y1	gb|BG322017	2e-35

C. elegans gene: W09B6.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687083533	gb|BM606557	2e-48
Anopheles gambiae	17000687439275	gb|BM618618	2e-46
Anopheles gambiae	17000687321659	gb|BM587799	2e-43
Anopheles gambiae	17000687086088	gb|BM608168	5e-42
Anopheles gambiae	17000687315677	gb|BM645103	2e-38
Anopheles gambiae	17000687236701	gb|BM579048	3e-33
Anopheles gambiae	17000659179265	gb|BM610911	5e-30
Ancylostoma caninum	pb31a01.y1	gb|BQ125044	2e-61
Ascaris suum	kh05g07.y1	gb|BI782124	2e-47
Ascaris suum	kh06f09.y1	gb|BI782194	4e-47
Ascaris suum	kk05h08.y1	gb|BQ095491	4e-43
Ascaris suum	kh01h12.y1	gb|BI781835	7e-42
Necator americanus	Na_L3_34C04_SAC	gb|BU666204	2.e-15
Strongyloides ratti	ku15c12.y1	gb|BQ091288	7e-29
Strongyloides stercoralis	kq36e08.y1	gb|BE580061	8e-43
Strongyloides stercoralis	kq60a06.y1	gb|BF015002	4e-38
Strongyloides stercoralis	kq52b10.y1	gb|BE581778	9e-31
Toxocara canis	ko08f10.y1	gb|BM966530	1e-37
Toxocara canis	ko24c07.y1	gb|BQ089283	1e-37

C. elegans gene: T19B10.2

Species	EST ID	Assession Number	E value
Brugia malayi	SW3D9CA428SK	gb|AA585672	1e-63
Haemonchus contortus	Hc_d11_28B01_SKPL	gb|BF423321	6e-55
Onchocerca volvulus	SWOv3MCAM47D12SK	gb|BF482074	2e-48
Onchocerca volvulus	SWOvAFCAP42G02SK	gb|AW600024	8e-57
Onchocerca volvulus	SWOvAFCAP37H10SK	gb|AW562321	2e-50
Onchocerca volvulus	SWOv3MCAM04C01SK	gb|AI053004	3e-40
Onchocerca volvulus	SWOv3MCAM33B12SK	gb|AW288189	5e-24
Pristionchus pacificus	rs17d11.y1	gb|AI986817	2e-61
Strongyloides ratti	kt46c03.y3	gb|BI323886	2e-57
Strongyloides ratti	kt76d02.y3	gb|BI502537	2e-57
Strongyloides ratti	kt15h05.y1	gb|BG893826	3e-49
Strongyloides ratti	kt64a08.y1	gb| BI323577	9e-12
Strongyloides stercoralis	kq07e10.y1	gb|BG227479	3e-67
Strongyloides stercoralis	kp97f11.y1	gb|BG227146	1e-59
Strongyloides stercoralis	kq43c11.y1	gb|BE581183	1e-57
Strongyloides stercoralis	kq51c07.y1	gb|BE581720	1e-36
Trichinella spiralis	ps16e05.y2	gb|BG520446	2e-12

C. elegans gene: F40G9.1

Species	EST ID	Assession Number	E value
Ancylostoma caninum	pk22g03.x1	gb|CA341524	3e-37
Ascaris suum	As_nc_09H02_SKPL	gb|BI594288	8e-29
Apis mellifera	BB160019B20G12.5	gb|BI515617	3e-10
Necator americanus	Na_L3_03F10_SAC	gb|BG4678493e-13	

C. elegans gene: M88.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000659338584	gb|BM622660	1e-16
Anopheles gambiae	17000687147208	gb|BM615535	5e-16
Anopheles gambiae	17000687312380	gb|BM643448	3e-14
Anopheles gambiae	17000687503711	gb|BM632047	2e-12
Anopheles gambiae	17000687317758	gb|BM646000	1e-11
Anopheles gambiae	17000687566305	gb|BM638257	1e-11
Anopheles gambiae	17000687507490	gb|BM633604	1e-10
Anopheles gambiae	17000687446031	gb|BM621183	2e-10
Anopheles gambiae	17000687490365	gb|BM597156	3e-10
Anopheles gambiae	17000687556043	gb|BM634822	7e-10
Anopheles gambiae	17000687507788	gb|BM633679	9e-10
Apis mellifera	BB170030A10B11.5	gb|BI505904	2e-12
Apis mellifera	BB170016A20D05.5	gb|BI510550	5e-08
Bombyx mori	AV403012	dbj|AV403012	4e-11
Bombyx mori	AV400933	dbj|AV400933	3e-10
Meloidogyne arenaria	rm24h02.y1	gb|BI746256	3e-15
Meloidogyne javanica	rk43d08.y1	gb|BG735742	5e-14
Meloidogyne javanica	rk97f05.y1	gb|BI745212	2e-12

C. elegans gene: CD4.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687371664	gb|BM650501	6e-67
Anopheles gambiae	17000687322329	gb|BM588180	3e-62
Anopheles gambiae	17000687071573	gb|BM602678	3e-60
Anopheles gambiae	17000687068376	gb|BM601670	7e-60
Anopheles gambiae	17000687313631	gb|BM644198	2e-56
Anopheles gambiae	17000687439860	gb|BM619034	2e-56
Anopheles gambiae	17000687277359	gb|BM583000	2e-55
Anopheles gambiae	17000687619748	gb|BM598398	5e-47
Artemia franciscana	ar10-065	gb|BQ605277	1e-63
Amblyomma variegatum	EST576373	gb|BM289839	2e-69
Bombyx mori	AV398746	dbj|AV398746	1e-61
Globodera rostochiensis	rr26d03.y1	gb|BM355545	2e-67
Heterodera glycines	ro21g02.y1	gb|BF014394	2e-68
Meloidogyne javanica	rk14h04.y1	gb|BE578613	4e-61
Meloidogyne arenaria	rm38a11.y1	gb|BI747271	8e-53
Meloidogyne hapla	rc34d01.y1	gb|BM902290	6e-51
Meloidogyne javanica	rk70b10.y1	gb|BI143067	1e-45
Globodera pallida	OP20486	gb|BM415412	3e-72
Pristionchus pacificus	rs39e05.y1	gb|AW097083	2e-76
Strongyloides ratti	kt29h05.y1	gb|BI073353	6e-69
Trichinella spiralis	pt02c08.y1	gb|BQ692053	3e-63
Trichinella spiralis	ps98a12.y1	gb|BQ542423	2e-47

C. elegans gene: F52B11.3

Species	EST ID	Assession Number	E value
Brugia malayi	MBAFCW3E10T3	gb|AA661399	4e-48
Meloidogyne hapla	rc57a01.y1	gb|BM952243	9e-71
Meloidogyne hapla	rc58e09.y1	gb|BQ090007	4e-56
Meloidogyne hapla	rc26c02.y1	gb|BM901200	4e-06
Meloidogyne arenaria	rm36a05.y1	gb|BI747105	7e-06
Meloidogyne arenaria	rm03h03.y1	gb|BI501693	7e-06
Strongyloides ratti	kt15h10.y1	gb|BG893830	7e-80
Strongyloides stercoralis	kq38b02.y1	gb|BE580180	1e-73
Strongyloides stercoralis	kq61e08.y1	gb|BF015258	2e-72
Strongyloides stercoralis	kq24d08.y1	gb|BE579237	1e-53
Strongyloides stercoralis	kq42f04.y1	gb|BE581128	1e-43
Strongyloides stercoralis	kp96c07.y1	gb|BG227056	8e-37

C. elegans gene: F41H10.7

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687316343	gb|BM645334	5e-20
Ancylostoma caninum	pb13c07.y1	gb|BM077653	2e-40
Ancylostoma caninum	pj06b02.y1	gb|BM130528	2e-40
Ancylostoma caninum	pj13h01.y1	gb|BM131118	5e-40
Ancylostoma caninum	pj01g12.y1	gb|BI704649	3e-34
Ancylostoma caninum	pj05b02.y1	gb|BM130446	4e-25
Ascaris suum	kk23a03.y2	gb|BQ381206	1e-48
Ascaris suum	kk23a11.y2	gb|BQ381214	6e-47
Ascaris suum	kj50f05.y1	gb|BM515548	7e-35
Ascaris suum	As_adfo_05B04_T3	gb|CA303612	7e-35
Ascaris suum	ki05a11.y1	gb|BM280791	4e-34
Ascaris suum	kj52c04.y1	gb|BM515678	3e-34
Ascaris suum	As_adfg_09B10_T3	gb|BU605554	1e-34
Ascaris suum	ki44g05.y1	gb|BM283072	2e-33
Ascaris suum	kj49f11.y1	gb|BM515471	4e-33
Ascaris suum	kj96b03.y1	gb|BQ095112	4e-33
Ascaris suum	kj96c04.y1	gb|BQ095124	1e-33
Ascaris suum	As_adfo_07A06_T3	gb|CA303746	1e-32
Ascaris suum	kh96b06.y1	gb|BM285220	2e-30
Ascaris suum	ki71h03.y1	gb|BM319371	2e-15
Brugia malayi	SWMFCA2329SK	gb|AA545829	9e-40
Brugia malayi	SWBmL3SBH11E05SK	gb|AI079048	3e-28
Brugia malayi	SWMFCA2496SK	gb|AA563533	3e-26
Globodera rostochiensis	rr17f12.y1	gb|BM354846	2e-53

Haemonchus contortus	pw07g07.y1	gb|CA034206	2e-34
Haemonchus contortus	pw07g09.y1	gb|CA034208	2e-34
Haemonchus contortus	pw14b10.y1	gb|CA033681	2e-33
Haemonchus contortus	pw10e09.y1	gb|CA034357	1e-29
Meloidogyne javanica	rk65e12.y1	gb|BG737014	5e-29
Meloidogyne javanica	rk98g01.y1	gb|BI745300	1e-28
Meloidogyne javanica	rk74d11.y1	gb|BI142820	1e-22
Meloidogyne hapla	rf67e06.y1	gb|BU094358	6e-20
Meloidogyne hapla	rc35e08.y1	gb|BM902404	2e-12
Meloidogyne hapla	rf86e02.y2	gb|BU095464	2e-12
Meloidogyne incognita	rb10d11.y1	gb|BM881480	6e-10
Globodera pallida	OP20484	gb|BM415410	2e-28
Necator americanus	Na_L3_24H05_SAC	gb|BU087819	2e-14
Onchocerca volvulus	SWOv3MCAM49H12SK	gb|BF599190	2e-61
Onchocerca volvulus	SWOvL3CAN71H05SK	gb|BF154352	8e-25
Onchocerca volvulus	SWOvAFCAP15C07SK	gb|AI308680	2e-49
Onchocerca volvulus	SWOvAFCAP27H07SK	gb|AI539947	1e-22
Onchocerca volvulus	SWOv3MCAM36B09SK	gb|AW308544	9e-17
Onchocerca volvulus	SWOv3MCA1157SK	gb|AI045995	6e-38
Parastrongyloides trichosuri	kx61d04.y1	gb|BM356240	6e-20
Parastrongyloides trichosuri	kx68g05.y1	gb|BM346265	1e-19
Parastrongyloides trichosuri	kx77d07.y1	gb|BM512626	1e-19
Parastrongyloides trichosuri	kx81e05.y1	gb|BM512881	1e-19
Parastrongyloides trichosuri	kx98d10.y2	gb|BM513719	1e-16
Parastrongyloides trichosuri	ky01b10.y1	gb|BM514338	1e-16
Parastrongyloides trichosuri	ky01b10.y3	gb|BQ274049	1e-16

Parastrongyloides trichosuri	kx76b10.y1	gb|BM812691	7e-11
Pristionchus pacificus	rs36h03.y1	gb|AW052554	3e-30
Pristionchus pacificus	rs32b04.y1	gb|AW114333	2e-27
Pristionchus pacificus	rs08h02.r1	gb|AA191857	5e-16
Strongyloides ratti	kt24a11.y3	gb|BI397325	7e-11

C. elegans gene: ZK783.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687050491	gb|BM599848	7e-17
Anopheles gambiae	17000687313297	gb|BM643943	1e-16
Apis mellifera	BB170008A10D11.5	gb|BI507755	2e-17
Bombyx mori	AV403913	dbj|AV403913	1e-18
Bombyx mori	AV405815	dbj|AV405815	9e-17

C. elegans gene: W10G6.3

Species	EST ID	Assession Number	E value
Ascaris suum	ki02g09.y1	gb|BM280603	1e-84
Ascaris suum	kh29h03.y1	gb|BI784031	3e-74
Ascaris suum	kj92f03.y1	gb|BM965152	1e-72
Ascaris lumbricoides	Al_am_44B09_T3	gb|BU586964	3e-67
Ascaris suum	ki08f11.y1	gb|BM281039	1e-65
Ascaris suum	kh97g02.y1	gb|BM284670	5e-63
Ascaris suum	kh67d02.y1	gb|BM033773	1e-62
Ascaris suum	kk52b05.y1	gb|BQ382546	9e-62
Ascaris suum	As_L3_09B01_SKPL	gb|BI594018	3e-61
Ascaris suum	kh07f03.y1	gb|BI782261	6e-60
Ascaris suum	kk55f09.y1	gb|BQ382765	2e-60
Ascaris suum	As_nc_20E04_SKPL	gb|BI594703	7e-56
Ascaris lumbricoides	Al_am_39H04_T3	gb|BU586869	6e-48
Ascaris suum	kh23d05.y1	gb|BI784404	1e-47
Ascaris suum	As_nc_10C07_SKPL	gb|BI594311	8e-47
Ascaris suum	As_adfo_18A08_T3	gb|CA304479	7e-46
Ascaris suum	MBAsBWA018M13R	gb|AW165649	1e-44
Ascaris suum	kj97h10.y1	gb|BQ095255	2e-44
Ascaris suum	kj91c09.y1	gb|BM965046	2e-42
Ascaris suum	As_nc_07A04_SKPL	gb|BI594184	6e-39
Ascaris suum	kj10g11.y1	gb|BM567150	4e-37
Ascaris suum	As_nc_17F06_SKPL	gb|BI594620	3e-37
Ascaris suum	ki07a10.y1	gb|BM280930	4e-34
Ascaris lumbricoides	Al_am_19E10_T3	gb|BU585851	2e-29
Ascaris suum	kj16d10.y1	gb|BM567546	1e-29
Brugia malayi	SWYD25CAU14E02SK	gb|AW675831	2e-75
Brugia malayi	SWMFCA1385SK	gb|AA231989	8e-50
Brugia malayi	MB3D6V8E10T3	gb|AA841889	1e-49
Brugia malayi	SWYACAL08E03SK	gb|BE758356	6e-45
Brugia malayi	SW3ICA2430SK	gb|AA255390	3e-42
Brugia malayi	MB3D6V8A06T3	gb|AA841843	1e-35

Brugia malayi	KJBmL3SZ4B22SK	gb|AI944353	2e-30
Brugia malayi	RRAMCA1524SK	gb|AA430804	2e-29
Dirofilaria immitis	ke22g11.y1	gb|BQ455787	1e-35
Globodera rostochiensis	rr58b08.y1	gb|BM344699	3e-78
Globodera rostochiensis	rr65c04.y1	gb|BM345560	5e-75
Globodera rostochiensis	rr30c09.y1	gb|BM355843	2e-59
Globodera rostochiensis	rr30a02.y1	gb|BM355821	3e-52
Haemonchus contortus	Hc_d11_11E10_SKPL	gb|BF060126	5e-57
Haemonchus contortus	Hc_d11_18E03_SKPL	gb|BF422872	1e-56
Litomosoides sigmodontis	JALsL3C179SAC	gb|AW152844	1e-74
Meloidogyne incognita	rd08a12.y1	gb|BQ613497	1e-68
Meloidogyne incognita	rd19e10.y1	gb|BQ613722	1e-68
Meloidogyne hapla	rc49c01.y1	gb|BM901834	6e-66
Meloidogyne hapla	rc26d08.y1	gb|BM901218	9e-65
Meloidogyne hapla	rc37g03.y1	gb|BM902598	6e-64
Meloidogyne incognita	rd02c03.y1	gb|BQ613170	3e-64
Meloidogyne hapla	rc42h03.y1	gb|BM900907	3e-62
Meloidogyne hapla	rf48d08.y1	gb|BQ836630	4e-62
Meloidogyne arenaria	rm47f07.y1	gb|BI747934	8e-53
Meloidogyne arenaria	rm28c11.y1	gb|BI746528	1e-48
Meloidogyne javanica	rk75h03.y1	gb|BI142900	3e-44
Onchocerca volvulus	SWOvAFCAP49B12SK	gb|BF199444	2e-62
Onchocerca volvulus	SWOv3MCAM52D01SK	gb|BF824665	1e-58
Onchocerca volvulus	SWOv3MCAM51A02SK	gb|BF727562	4e-58
Onchocerca volvulus	SWOvL2CAS06B03SK	gb|AW980259	3e-77
Onchocerca volvulus	SWOvAFCAP02E12SK	gb|AI077021	7e-73
Onchocerca volvulus	SWOv3MCAM26G09SK	gb|AI670483	5e-59
Onchocerca volvulus	SWOvL2CAS03E05SK	gb|AI444905	9e-50
Onchocerca volvulus	SWOv3MCAM07B07SK	gb|AI317899	7e-46
Onchocerca volvulus	SWOvAFCB315SK	gb|AI815264	2e-82
Onchocerca volvulus	SWOvL3CAN13E07	gb|AA917260	2e-51
Onchocerca volvulus	SWOv3MCA822SK	gb|AA294585	2e-51
Ostertagia ostertagi	ph53g02.y1	gb|BM896621	6e-77

Ostertagia ostertagi	ph69a09.y1	gb|BQ099825	3e-43
Parastrongyloides trichosuri	kx18a11.y3	gb|BI322222	1e-43
Strongyloides ratti	kt51c06.y4	gb|BI742464	8e-50
Strongyloides stercoralis	kp60g10.y1	gb|BE224367	7e-43
Strongyloides stercoralis	kp89h11.y1	gb|BG226499	5e-35
Strongyloides stercoralis	kq58d04.y1	gb|BF014961	2e-66
Strongyloides stercoralis	kq16d05.y1	gb|BG227868	9e-59
Strongyloides stercoralis	kq25d02.y1	gb|BE579290	2e-52
Strongyloides stercoralis	kq07e05.y1	gb|BG227475	3e-50
Strongyloides stercoralis	kq38a11.y1	gb|BE580177	5e-50
Strongyloides stercoralis	kq01b02.y1	gb|BG226921	4e-46
Strongyloides stercoralis	kq43f12.y1	gb|BE581211	3e-45
Strongyloides stercoralis	kq59e08.y1	gb|BF014970	8e-41
Strongyloides stercoralis	kq31d11.y1	gb|BE579614	4e-34
Strongyloides stercoralis	kq17c02.y1	gb|BG227920	3e-30
Toxocara canis	ko17e01.y1	gb|BM965806	1e-52
Trichinella spiralis	ps41c08.y1	gb|BG353660	6e-68
Trichinella spiralis	ps21c11.y4	gb|BG732010	2e-66
Trichuris muris	Tm_ad_02F09_SKPL	gb|BF049882	2e-69
Trichuris muris	Tm_ad_32C10_SKPL	gb|BM174670	3e-69
Trichuris muris	Tm_ad_34B05_SKPL	gb|BM174819	3e-41
Trichuris muris	Tm_ad_28B10_SKPL	gb|BM174335	2e-38
Trichuris muris	Tm_ad_41B01_SKPL	gb|BM277502	4e-34
Trichuris muris	Tm_ad_30G11_SKPL	gb|BM174554	4e-30

C. elegans gene: C17G1.6

Species	EST ID	Assession Number	E value
Ancylostoma caninum	pb60d09.y1	gb|BQ667369	3e-21
Ancylostoma caninum	pj59h02.y3	gb|BU780981	3e-17
Ascaris suum	kk63b06.y1	gb|BQ835552	8e-41
Ascaris suum	kk75f04.y1	gb|BU965942	5e-41
Ascaris suum	kk67a07.y1	gb|BQ835133	5e-39
Ascaris suum	kk81d05.y1	gb|BU966321	5e-39
Ascaris suum	kk82h03.y1	gb|BU966423	1e-22
Bombyx mori	AU002182	dbj|AU002182	2e-18
Brugia malayi	MBAFCX3H02T3	gb|AA471557	5e-17
Meloidogyne arenaria	rm39h08.y1	gb|BI747415	2e-17
Meloidogyne arenaria	rm44a01.y1	gb|BI747765	4e-16
Necator americanus	Na_L3_54E05_SAC	gb|BU089288	2e-29
Necator americanus	Na_L3_46G04_SAC	gb|BU088646	7e-27
Necator americanus	Na_L3_33B12_SAC	gb|BU088268	3e-26
Necator americanus	Na_L3_42D01_SAC	gb|BU666771	2e-25
Necator americanus	Na_L4_01D08_SAC	gb|BG467914	2e-24
Necator americanus	Na_L3_43E08_SAC	gb|BU666872	5e-20
Necator americanus	Na_L3_28E03_SAC	gb|BU088135	1e-19
Necator americanus	Na_L3_18C11_SAC	gb|BU087246	3e-18
Necator americanus	Na_L3_23G12_SAC	gb|BU087729	1e-16
Ostertagia ostertagi	Oo_ad_01F02_LambdaGT11FO	gb|BG733933	6e-20
Ostertagia ostertagi	ph69g06.y1	gb|BQ099886	8e-18
Ostertagia ostertagi	ph37c02.y1	gb|BM897683	1e-17
Ostertagia ostertagi	ph43h10.y1	gb|BM897848	1e-17
Ostertagia ostertagi	ph47c08.y1	gb|BM896734	4e-17
Ostertagia ostertagi	ph38g05.y1	gb|BM897764	2e-16
Ostertagia ostertagi	ph44f10.y1	gb|BM897904	1e-15
Parastrongyloides trichosuri	kx10f03.y3	gb|BI451087	2e-34
Parastrongyloides trichosuri	kx16e12.y3	gb|BI322818	6e-29
Parastrongyloides trichosuri	kx13e07.y3	gb|BI322556	9e-22
Parastrongyloides trichosuri	kx16d11.y3	gb|BI322807	1e-18

Parastrongyloides trichosuri	kx34b01.y1	gb|BI742691	1e-18
Parastrongyloides trichosuri	kx27f02.y1	gb|BI501067	1e-17
Parastrongyloides trichosuri	kx35c07.y1	gb|BI742807	1e-17
Parastrongyloides trichosuri	kx42f06.y1	gb|BI743922	1e-15
Parastrongyloides trichosuri	kx26a10.y1	gb|BI500947	4e-15
Pristionchus pacificus	rs82e09.y1	gb|BI500840	2e-23
Pristionchus pacificus	rt09b02.y1	gb|BQ087806	7e-19
Pristionchus pacificus	rs73h01.y1	gb|BI500514	1e-15
Pristionchus pacificus	rs36b09.y1	gb|AW052495	5e-20
Strongyloides ratti	kt84a03.y1	gb|BI741990	3e-39
Strongyloides ratti	kt65c12.y1	gb|BI323632	3e-21
Strongyloides ratti	kt22h07.y1	gb|BG894012	8e-18
Strongyloides ratti	ku07c07.y1	gb|BM879025	6e-16
Strongyloides stercoralis	kp47c05.y1	gb|BG224501	9e-35
Strongyloides stercoralis	kp25g06.y1	gb|BE029170	3e-33
Strongyloides stercoralis	kp60b07.y1	gb|BE224326	5e-33
Strongyloides stercoralis	kp60f02.y1	gb|BE224353	8e-28
Strongyloides stercoralis	kp04a10.y1	gb|AW496628	7e-25
Strongyloides stercoralis	kp05g07.y1	gb|AW496678	5e-25
Strongyloides stercoralis	kp24d04.y1	gb|BE029064	1e-25
Strongyloides stercoralis	kp36h12.y1	gb|BE029947	3e-25
Strongyloides stercoralis	kp54h12.y1	gb|BE224535	2e-25
Strongyloides stercoralis	kp85d05.y1	gb|BE223897	9e-25
Strongyloides stercoralis	kp50g09.y1	gb|BG224805	3e-25
Strongyloides stercoralis	kp48d11.y1	gb|BG224592	4e-25
Strongyloides stercoralis	kp40f09.y1	gb|BE030258	6e-24
Strongyloides stercoralis	kp23b01.y1	gb|BE028980	8e-23
Strongyloides stercoralis	kp53g03.y1	gb|BE224009	8e-23
Strongyloides stercoralis	kp73g06.y1	gb|BE223185	5e-23
Strongyloides stercoralis	kp84c04.y2	gb|BE581022	2e-23
Strongyloides stercoralis	kp45a04.y1	gb|BG224325	8e-23
Strongyloides stercoralis	kp26g12.y1	gb|BE029255	4e-22
Strongyloides stercoralis	kp78d11.y2	gb|BE579761	2e-22

Strongyloides stercoralis	kp35h02.y1	gb|BE029861	2e-21
Strongyloides stercoralis	kp09g10.y1	gb|AW587924	4e-20
Strongyloides stercoralis	kp26f12.y1	gb|BE029245	4e-20
Strongyloides stercoralis	kp54d06.y1	gb|BE224068	2e-20
Strongyloides stercoralis	kp85b01.y1	gb|BE223873	1e-20
Strongyloides stercoralis	kp49d06.y1	gb|BG224673	2e-20
Strongyloides stercoralis	kp58g05.y1	gb|BE224258	2e-19
Strongyloides stercoralis	kp44a03.y1	gb|BG225948	1e-19
Strongyloides stercoralis	kp66h07.y1	gb|BG225405	7e-19
Strongyloides stercoralis	kp29d05.y1	gb|BE029604	6e-18
Strongyloides stercoralis	kp39g02.y1	gb|BE030188	3e-18
Strongyloides stercoralis	kp41f04.y1	gb|BE030329	2e-18
Strongyloides stercoralis	kp55e05.y1	gb|BE224547	5e-18
Strongyloides stercoralis	TNSSFH0001	gb|N21795	3e-18
Strongyloides stercoralis	kp29g02.y1	gb|BE029626	7e-17
Strongyloides stercoralis	kp63g05.y1	gb|BE224503	7e-17
Strongyloides stercoralis	kp80b11.y2	gb|BE580669	7e-17
Strongyloides stercoralis	kp86b04.y1	gb|BE223626	9e-17
Strongyloides stercoralis	kp65e09.y1	gb|BG225224	4e-17
Strongyloides stercoralis	kp25d11.y1	gb|BE029146	6e-16
Strongyloides stercoralis	kp34d10.y1	gb|BE029758	2e-16
Strongyloides stercoralis	kp34e03.y1	gb|BE029762	3e-16
Strongyloides stercoralis	kp37b04.y1	gb|BE029959	2e-16
Strongyloides stercoralis	kp57h08.y1	gb|BE224605	6e-16
Strongyloides stercoralis	kp58e10.y1	gb|BE224244	6e-16
Strongyloides stercoralis	kp60e04.y1	gb|BE224345	7e-16
Strongyloides stercoralis	kp61b01.y1	gb|BG225005	2e-16
Strongyloides stercoralis	kp48f04.y1	gb|BG224608	3e-16
Strongyloides stercoralis	kp72b08.y1	gb|BG225809	3e-16
Strongyloides stercoralis	kp03g10.y1	gb|AW496617	3e-15
Strongyloides stercoralis	kp22h08.y1	gb|BE028968	1e-15
Strongyloides stercoralis	kp71d04.y1	gb|BG225739	1e-15
Strongyloides stercoralis	kp71c12.y1	gb|BG225735	3e-15

Strongyloides stercoralis	kp61e11.y1	gb|BG225048	4e-15
Strongyloides stercoralis	kq08h10.y1	gb|BG226082	3e-29
Trichinella spiralis	pt31e04.y1	gb|BQ738378	2e-17

C. elegans gene: T05C12.10

Species	EST ID	Assession Number	E value
Meloidogyne incognita	rd30d09.y1	gb|BQ613344	7e-47
Meloidogyne incognita	rb25a03.y1	gb|BM882030	3e-17
Onchocerca volvulus	SWOv3MCAM30H08SK	gb|AW257707	1e-22
Onchocerca volvulus	SWOv3MCAM21D03SK	gb|AI444860	2e-12
Strongyloides stercoralis	kq58h02.y1	gb|BF014893	1e-34
Strongyloides stercoralis	kq19a09.y1	gb|BG226231	2e-24
Strongyloides stercoralis	kq23h04.y1	gb|BE579200	3e-22

C. elegans gene: R05D11.3

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687042995	gb|BM599374	2e-28
Anopheles gambiae	17000687072218	gb|BM603030	2e-28
Anopheles gambiae	17000687443914	gb|BM593861	2e-28
Anopheles gambiae	17000687314705	gb|BM586411	2e-27
Anopheles gambiae	17000687162185	gb|BM576239	1e-26
Anopheles gambiae	17000687478579	gb|BM623382	3e-23
Anopheles gambiae	17000687489887	gb|BM623861	3e-23
Ascaris lumbricoides	Al_am_06F12_T3	gb|BU585500	4e-44
Ascaris suum	ki56a07.y1	gb|BM281377	3e-41
Ascaris suum	MBAsBWA194M13R	gb|AW165779	6e-38
Ascaris lumbricoides	Al_am_08A06_T3	gb|BU585565	4e-32
Ascaris suum	kj45g01.y2	gb|BM517341	1e-31
Ascaris suum	ki47g06.y1	gb|BM283275	4e-29
Ascaris lumbricoides	Al_am_28D07_T3	gb|BU586336	1e-22
Apis mellifera	BB160006B20G02.5	gb|BI511717	7e-29
Apis mellifera	BB160015A20D12.5	gb|BI514405	7e-29
Apis mellifera	BB170018A10D07.5	gb|BI509477	7e-29
Bombyx mori	AU004592	dbj|AU004592	2e-27
Bombyx mori	AU006081	dbj|AU006081	6e-27
Bombyx mori	AV406293	dbj|AV406293	6e-27
Bombyx mori	AV404938	dbj|AV404938	5e-24
Globodera rostochiensis	rr58f12.y1	gb|BM344746	3e-41
Heterodera glycines	ro22c04.y1	gb|BF014168	4e-39
Heterodera glycines	ro27a03.y1	gb|BF014695	1e-34

Meloidogyne hapla	rc70d01.y1	gb|BQ125588	8e-38
Ostertagia ostertagi	ph86a03.y1	gb|BQ625869	2e-39
Pristionchus pacificus	rs32b08.y1	gb|AW114337	6e-41
Strongyloides ratti	ku24e01.y1	gb|BQ091075	7e-44
Strongyloides stercoralis	kp53g06.y1	gb|BE224012	4e-44
Strongyloides stercoralis	kp46d05.y1	gb|BG224431	4e-44
Strongyloides stercoralis	kp49e01.y1	gb|BG224680	4e-44
Strongyloides stercoralis	kp26a06.y1	gb|BE029191	3e-43
Trichuris muris	Tm_ad_12H09_SKPL	gb|BG577863	2e-24

C. elegans gene: C42D8.5

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687312136	gb|BM586309	1e-24
Anopheles gambiae	17000687442312	gb|BM593385	1e-20
Anopheles gambiae	17000687147222	gb|BM615549	3e-20
Anopheles gambiae	17000687284463	gb|BM640720	6e-20
Anopheles gambiae	17000687388771	gb|BM591666	6e-20
Anopheles gambiae	17000687076415	gb|BM605493	1e-18
Anopheles gambiae	17000687306814	gb|BM641014	1e-17
Anopheles gambiae	17000687321320	gb|BM587522	1e-16
Anopheles gambiae	17000687140860	gb|BM614061	5e-14
Anopheles gambiae	17000687383033	gb|BM590327	9e-13
Anopheles gambiae	17000668455074	gb|BM592015	8e-11
Anopheles gambiae	17000687446260	gb|BM595069	6e-06
Anopheles gambiae	17000687498456	gb|BM628360	2e-05
Anopheles gambiae	17000687317872	gb|BM646088	3e-04
Amblyomma variegatum	EST576720	gb|BM290186	1e-23
Apis mellifera	BB170014A10G05.5	gb|BI509028	9e-26
Apis mellifera	BB170005B10F11.5	gb|BI504652	4e-13
Apis mellifera	BB170029A10E09.5	gb|BI509998	8e-11
Bombyx mori	AU004618	dbj|AU004618	2e-17
Bombyx mori	AU005275	dbj|AU005275	2e-11
Bombyx mori	AU004718	dbj|AU004718	4e-06
Manduca sexta	EST816	gb|BE015590	3e-20
Meloidogyne arenaria	rm04g06.y1	gb|BI501765	4e-41
Meloidogyne incognita	rb11d01.y1	gb|BM881559	8e-41

Meloidogyne incognita	rb18a12.y1	gb|BM880769	3e-41
Meloidogyne incognita	rb26b04.y1	gb|BM882125	7e-40
Meloidogyne javanica	rk44e09.y1	gb|BG735807	6e-38
Meloidogyne arenaria	rm47b10.y1	gb|BI747899	2e-35
Meloidogyne incognita	rb26c05.y1	gb|BM882137	2e-35
Meloidogyne hapla	rc34h03.y1	gb|BM902335	9e-26
Parastrongyloides trichosuri	kx21e10.y1	gb|BI451241	6e-33
Pristionchus pacificus	rs54d09.y1	gb|AW114662	3e-39
Trichinella spiralis	ps52g05.y1	gb|BG520845	1e-15
Trichuris muris	Tm_ad_35E08_SKPL	gb|BM277122	6e-15
Trichuris muris	Tm_ad_31D02_SKPL	gb|BM174595	9e-13

C. elegans gene: ZK430.8

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687491429	gb|BM624505	3e-25
Anopheles gambiae	17000687503479	gb|BM597766	9e-23
Anopheles gambiae	17000687144729	gb|BM614748	2e-22
Anopheles gambiae	17000659431849	gb|BM584934	2e-21
Anopheles gambiae	17000687475690	gb|BM595494	8e-21
Anopheles gambiae	17000687085569	gb|BM607770	2e-20
Anopheles gambiae	17000687385695	gb|BM655137	4e-19
Aedes aegypti	AEMTAN84	gb|AI650118	1e-22
Aedes aegypti	AEMTBE10	gb|AI657546	2e-21
Amblyomma variegatum	EST576420	gb|BM289886	2e-47
Amblyomma variegatum	EST576491	gb|BM289957	1e-45
Bombyx mori	AU005825	dbj|AU005825	1e-24
Brugia malayi	BSBmL3SZ44P22SK	gb|AI723670	8e-40
Brugia malayi	SWAMCAC30E11SK	gb|AI784735	3e-26
Brugia malayi	SWAMCA791SK	gb|W69058	2e-19
Heterodera glycines	ro60g11.y3	gb|BI396718	1e-27
Meloidogyne hapla	rc06f11.y1	gb|BM883419	1e-36
Strongyloides ratti	kt33e05.y1	gb|BI073673	5e-31
Strongyloides stercoralis	kq05g08.y1	gb|BG227360	5e-72
Trichinella spiralis	ps41e07.y1	gb|BG353679	3e-28

C. elegans gene: W08F4.6

Species	EST ID	Assession Number	E value
Brugia malayi	SWYACAL11B04SK	gb|BE758466	e-104
Brugia malayi	SWYD25CAU09E12SK	gb|AW352455	2e-93
Brugia malayi	SWYACAL10F05SK	gb|BE758438	1e-86
Brugia malayi	SWMFCA2071SK	gb|AA480716	1e-77
Brugia malayi	SWMFCA2926SK	gb|AA598365	1e-77
Brugia malayi	SWMFCA2164SK	gb|AA283595	9e-59
Brugia malayi	SWAMCA1093SK	gb|AA032101	1e-51
Brugia malayi	RRAMCA1520SK	gb|AA430774	2e-44
Brugia malayi	RRAMCA2132SK	gb|AI574633	6e-05
Onchocerca volvulus	SWOv3MCAM55E04SK	gb|BG310588	e-121
Onchocerca volvulus	SWOvAFCAP46H10SK	gb|BE949537	4e-97
Onchocerca volvulus	SWOv3MCAM54B10SK	gb|BF918270	1e-84
Onchocerca volvulus	SWOv3MCAM53A07SK	gb|BF824723	1e-74
Onchocerca volvulus	SWOv3MCAM51C05SK	gb|BF727588	3e-74
Onchocerca volvulus	SWOv3MCAM52D06SK	gb|BF824670	4e-64
Onchocerca volvulus	SWOv3MCAM51F03SK	gb|BF727618	2e-53
Onchocerca volvulus	SWOv3MCAM61A06SK	gb|BG809067	7e-52
Onchocerca volvulus	SWOvAFCAP49D03SK	gb|BF199456	9e-46
Onchocerca volvulus	SWOvAFCAP48B10SK	gb|BF064382	3e-39
Onchocerca volvulus	SWOvAFCAP35C02SK	gb|AW562139	1e-93
Onchocerca volvulus	SWOv3MCAM28D06SK	gb|AI692125	5e-91
Onchocerca volvulus	SWOvMfCAR10H04SK	gb|AW874896	1e-87
Onchocerca volvulus	SWOv3MCAM38A10SK	gb|AW313047	2e-75
Onchocerca volvulus	SWOvAFCAP34D11SK	gb|AW562114	1e-73

Onchocerca volvulus	SWOv3MCAM12D08SK	gb|AI322100	3e-72
Onchocerca volvulus	SWOv3MCAM38E03SK	gb|AW313086	6e-71
Onchocerca volvulus	SWOv3MCAM26F11SK	gb|AI670476	5e-63
Onchocerca volvulus	SWOvAFCAP16F04SK	gb|AI318006	1e-51
Onchocerca volvulus	SWOvAFCAP28E11SK	gb|AI540006	1e-50
Onchocerca volvulus	SWOvAFCAP35E04SK	gb|AW562163	1e-50
Onchocerca volvulus	SWOv3MCAM37F09SK	gb|AW313003	2e-50
Onchocerca volvulus	SWOv3MCAM23B03SK	gb|AI603814	5e-39
Onchocerca volvulus	SWOv3MCAM37E10SK	gb|AW312994	5e-35
Onchocerca volvulus	SWOvMfCAR04C04SK	gb|AI381166	9e-32
Onchocerca volvulus	SWOvAFCAP15A02SK	gb|AI771077	5e-31
Onchocerca volvulus	SWOvAFCAP25C08SK	gb|AI368292	4e-22
Onchocerca volvulus	SWOv3MCA1962SK	gb|AA618916	4e-97
Onchocerca volvulus	SWOv3MCA705SK	gb|AA294494	6e-61
Onchocerca volvulus	SWOv3MCA1335SK	gb|AA293981	3e-56
Onchocerca volvulus	SWOv3MCA107SK	gb|AA293944	4e-53
Onchocerca volvulus	SWOv3MCA1898SK	gb|AA618908	1e-20
Parastrongyloides trichosuri	kx60h05.y1	gb|BM346811	6e-89
Parastrongyloides trichosuri	kx72f10.y1	gb|BM513102	5e-55
Parastrongyloides trichosuri	kx76e12.y1	gb|BM812715	4e-47
Parastrongyloides trichosuri	kx23a05.y1	gb|BI451341	2e-31
Strongyloides stercoralis	kp97h03.y1	gb|BG227161	2e-84
Strongyloides stercoralis	kq65c07.y1	gb|BF015176	3e-66
Strongyloides stercoralis	kq38c10.y1	gb|BE580196	4e-65

C. elegans gene: C11H1.3

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687504402	gb|BM632519	8e-14
Ascaris suum	kj59a10.y1	gb|BM569296	2e-11
Brugia malayi	BSBmL3SZ15J6SK	gb|AI783191	7e-37
Brugia malayi	MBAFCW6C02T3	gb|AA842256	7e-19
Brugia malayi	BSBmL3SZ45E24SK	gb|AI723685	4e-10
Trichinella spiralis	ps40b05.y1	gb|BG353953	5e-11
Trichuris muris	Tm_ad_08A04_SKPL	gb|BG577585	1e-19

C. elegans gene: T23F2.1

Species	EST ID	Assession Number	E value
Meloidogyne incognita	rb19d10.y1	gb|BM880892	6e-65
Meloidogyne hapla	rc09a08.y1	gb|BM883631	1e-57
Meloidogyne hapla	rc19d06.y1	gb|BM884107	5e-57
Meloidogyne hapla	rc80b07.y1	gb|BQ627436	2e-57
Meloidogyne javanica	rk89h09.y1	gb|BI744669	3e-52
Ostertagia ostertagi	ph80a06.y1	gb|BQ457577	5e-36
Pristionchus pacificus	rs74f08.y1	gb|BI703617	4e-13
Pristionchus pacificus	rs73e09.y1	gb|BI703595	2e-10
Strongyloides stercoralis	kq31b05.y1	gb|BE579591	7e-75
Strongyloides stercoralis	kp96a08.y1	gb|BG227048	1e-22

C. elegans gene: R07E4.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687445042	gb|BM594887	3e-70
Amblyomma variegatum	EST574524	gb|BM291982	9e-68
Globodera rostochiensis	rr28f03.y1	gb|BM355711	3e-63
Meloidogyne hapla	rf47g07.y1	gb|BQ836585	9e-76
Meloidogyne hapla	rf44f11.y1	gb|BQ836331	4e-73
Meloidogyne arenaria	rm39a02.y1	gb|BI747341	5e-66
Meloidogyne hapla	rf37e06.y1	gb|BQ837060	4e-66
Meloidogyne hapla	rf45g11.y1	gb|BQ836426	4e-66
Meloidogyne hapla	rf50g04.y2	gb|BU094140	4e-66
Meloidogyne hapla	rf53g05.y2	gb|BU094227	4e-66
Meloidogyne hapla	rf58d10.y1	gb|BQ835832	4e-66
Meloidogyne hapla	rf67f06.y1	gb|BU094368	4e-66
Meloidogyne arenaria	rm02d02.y1	gb|BI501589	1e-65
Meloidogyne arenaria	rm02f05.y1	gb|BI501609	1e-65
Meloidogyne arenaria	rm02g08.y1	gb|BI501619	2e-65
Meloidogyne arenaria	rm02h06.y1	gb|BI501626	3e-65
Meloidogyne arenaria	rm03b06.y1	gb|BI501643	1e-65
Meloidogyne arenaria	rm03f11.y1	gb|BI501681	1e-65
Meloidogyne arenaria	rm04b02.y1	gb|BI501716	1e-65
Meloidogyne arenaria	rm04b09.y1	gb|BI501721	9e-65
Meloidogyne arenaria	rm04c09.y1	gb|BI501729	4e-65
Meloidogyne arenaria	rm04c12.y1	gb|BI501732	1e-65
Meloidogyne arenaria	rm04g02.y1	gb|BI501762	1e-65
Meloidogyne arenaria	rm04g05.y1	gb|BI501764	1e-65

Meloidogyne arenaria	rm05d02.y1	gb|BI501806	2e-65
Meloidogyne arenaria	rm06f06.y1	gb|BI501906	3e-65
Meloidogyne arenaria	rm07a04.y1	gb|BI501933	3e-65
Meloidogyne arenaria	rm07a08.y1	gb|BI501937	9e-65
Meloidogyne arenaria	rm07f06.y1	gb|BI501985	3e-65
Meloidogyne arenaria	rm13b12.y1	gb|BI862855	1e-65
Meloidogyne arenaria	rm14g07.y1	gb|BI862971	1e-65
Meloidogyne arenaria	rm16g11.y1	gb|BI863129	1e-65
Meloidogyne arenaria	rm17c08.y1	gb|BI745704	1e-65
Meloidogyne arenaria	rm18d04.y1	gb|BI745781	1e-65
Meloidogyne arenaria	rm18e04.y1	gb|BI745792	9e-65
Meloidogyne arenaria	rm19f11.y1	gb|BI745875	3e-65
Meloidogyne arenaria	rm21a07.y1	gb|BI745964	1e-65
Meloidogyne arenaria	rm21d04.y1	gb|BI745991	1e-65
Meloidogyne arenaria	rm23a02.y1	gb|BI746123	1e-65
Meloidogyne arenaria	rm23c11.y1	gb|BI746144	1e-65
Meloidogyne arenaria	rm26a06.y1	gb|BI746349	3e-65
Meloidogyne arenaria	rm28c08.y1	gb|BI746525	3e-65
Meloidogyne arenaria	rm29g05.y1	gb|BI746637	1e-65
Meloidogyne arenaria	rm30f07.y1	gb|BI746703	3e-65
Meloidogyne arenaria	rm31d04.y1	gb|BI746759	1e-65
Meloidogyne arenaria	rm31f01.y1	gb|BI746774	1e-65
Meloidogyne arenaria	rm32a08.y1	gb|BI746802	1e-65
Meloidogyne arenaria	rm33c02.y1	gb|BI746890	1e-65
Meloidogyne arenaria	rm35a06.y1	gb|BI747032	3e-65
Meloidogyne arenaria	rm35d09.y1	gb|BI747063	1e-65

Meloidogyne arenaria	rm37e04.y1	gb|BI747228	9e-65
Meloidogyne arenaria	rm39h04.y1	gb|BI747413	1e-65
Meloidogyne arenaria	rm40d11.y1	gb|BI747460	1e-65
Meloidogyne arenaria	rm40f08.y1	gb|BI747479	3e-65
Meloidogyne arenaria	rm41c05.y1	gb|BI747526	1e-65
Meloidogyne arenaria	rm45b05.y1	gb|BI747647	7e-65
Meloidogyne arenaria	rm45b12.y1	gb|BI747653	5e-65
Meloidogyne arenaria	rm45e12.y1	gb|BI747681	1e-65
Meloidogyne arenaria	rm45h12.y1	gb|BI747711	1e-65
Meloidogyne arenaria	rm46g06.y1	gb|BI747868	9e-65
Meloidogyne arenaria	rm47a11.y1	gb|BI747891	4e-65
Meloidogyne arenaria	rm47c05.y1	gb|BI747905	1e-65
Meloidogyne arenaria	rm47c09.y1	gb|BI747909	1e-65
Meloidogyne arenaria	rm47d03.y1	gb|BI747914	1e-65
Meloidogyne hapla	rf26f11.y1	gb|BQ837462	2e-65
Meloidogyne incognita	ra84f09.y1	gb|BM773674	9e-65
Meloidogyne incognita	ra84f12.y1	gb|BM773677	1e-65
Meloidogyne incognita	ra92d12.y1	gb|BM774355	1e-65
Meloidogyne incognita	ra93c08.y1	gb|BM774423	4e-65
Meloidogyne incognita	ra95b12.y1	gb|BM774573	1e-65
Meloidogyne incognita	ra96h04.y1	gb|BM774720	1e-65
Meloidogyne incognita	ra96h10.y1	gb|BM774726	9e-65
Meloidogyne incognita	ra99c02.y1	gb|BM882309	1e-65
Meloidogyne incognita	ra99d03.y1	gb|BM882321	1e-65
Meloidogyne incognita	ra99h05.y1	gb|BM882366	1e-65
Meloidogyne incognita	rb02h10.y1	gb|BM882545	9e-65

Meloidogyne incognita	rb03a01.y1	gb|BM882548	1e-65
Meloidogyne incognita	rb06c07.y1	gb|BM881126	7e-65
Meloidogyne incognita	rb08d08.y1	gb|BM881299	1e-65
Meloidogyne incognita	rb09d03.y1	gb|BM881380	9e-65
Meloidogyne incognita	rb11b10.y1	gb|BM881544	1e-65
Meloidogyne incognita	rb11f12.y1	gb|BM881592	1e-65
Meloidogyne incognita	rb12g03.y1	gb|BM881679	1e-65
Meloidogyne incognita	rb16b01.y1	gb|BM880596	1e-65
Meloidogyne incognita	rb19e08.y1	gb|BM880901	1e-65
Meloidogyne incognita	rb20d04.y1	gb|BM880267	1e-65
Meloidogyne incognita	rb23a12.y1	gb|BM880504	4e-65
Meloidogyne incognita	rb23g01.y1	gb|BM880561	9e-65
Meloidogyne incognita	rb24b04.y1	gb|BM881952	4e-65
Meloidogyne incognita	rb28d01.y1	gb|BM882671	1e-65
Meloidogyne javanica	rk45d01.y1	gb|BG735927	4e-65
Meloidogyne javanica	rk49a03.y1	gb|BG736042	5e-65
Meloidogyne javanica	rk49b09.y1	gb|BG736055	1e-65
Meloidogyne javanica	rk53a04.y1	gb|BG736196	4e-65
Meloidogyne javanica	rk53c10.y1	gb|BG736217	4e-65
Meloidogyne javanica	rk54h02.y1	gb|BG736324	4e-65
Meloidogyne javanica	rk57b01.y1	gb|BG736436	4e-65
Meloidogyne javanica	rk58f10.y1	gb|BG736536	4e-65
Meloidogyne javanica	rk66b08.y1	gb|BG737056	4e-65
Meloidogyne javanica	rk89g01.y1	gb|BI744652	5e-65
Meloidogyne arenaria	rm18g05.y1	gb|BI745808	3e-64
Meloidogyne arenaria	rm24a02.y1	gb|BI746195	1e-64

Meloidogyne arenaria	rm26h04.y1	gb|BI746420	2e-64
Meloidogyne arenaria	rm27c07.y1	gb|BI746449	2e-64
Meloidogyne arenaria	rm37e08.y1	gb|BI747232	3e-64
Meloidogyne arenaria	rm40h05.y1	gb|BI747498	2e-64
Meloidogyne arenaria	rm42g03.y1	gb|BI747618	3e-64
Meloidogyne incognita	ra83d08.y1	gb|BM773565	3e-64
Meloidogyne incognita	ra89e11.y1	gb|BM774108	1e-64
Meloidogyne incognita	ra90b04.y1	gb|BM774157	5e-64
Meloidogyne incognita	rb08b07.y1	gb|BM881275	5e-64
Meloidogyne incognita	rb12c06.y1	gb|BM881640	8e-64
Meloidogyne incognita	rb13f04.y1	gb|BM881755	5e-64
Meloidogyne incognita	rb14a12.y1	gb|BM881795	2e-64
Meloidogyne incognita	rb15b06.y1	gb|BM881885	3e-64
Meloidogyne incognita	rb20f11.y1	gb|BM880296	5e-64
Meloidogyne incognita	rb30a09.y1	gb|BM882822	2e-64
Meloidogyne incognita	rb30e01.y1	gb|BM882861	1e-64
Meloidogyne javanica	rk45f07.y1	gb|BG735952	1e-64
Meloidogyne javanica	rk53d07.y1	gb|BG736223	5e-64
Meloidogyne javanica	rk57h07.y1	gb|BG736497	1e-64
Meloidogyne javanica	rk60a03.y1	gb|BG736616	5e-64
Meloidogyne javanica	rk60e11.y1	gb|BG736654	5e-64
Meloidogyne javanica	rk62d09.y1	gb|BG736793	5e-64
Meloidogyne javanica	rk64h06.y1	gb|BG736964	5e-64
Meloidogyne javanica	rl01a06.y1	gb|BI863144	5e-64
Meloidogyne arenaria	rm05c01.y1	gb|BI501797	2e-63
Meloidogyne arenaria	rm34e11.y1	gb|BI746992	1e-63

Meloidogyne hapla	rc05b08.y1	gb|BM883283	2e-63
Meloidogyne hapla	rc08c12.y1	gb|BM883572	2e-63
Meloidogyne hapla	rc32b09.y1	gb|BM902099	2e-63
Meloidogyne hapla	rc42b02.y1	gb|BM900837	1e-63
Meloidogyne hapla	rc44g03.y1	gb|BM901068	1e-63
Meloidogyne hapla	rc47g08.y1	gb|BM901701	2e-63
Meloidogyne hapla	rc48e03.y1	gb|BM901766	1e-63
Meloidogyne hapla	rc49g09.y1	gb|BM901884	2e-63
Meloidogyne incognita	ra84b02.y1	gb|BM773624	5e-63
Meloidogyne incognita	ra84b07.y1	gb|BM773629	5e-63
Meloidogyne incognita	ra84g09.y1	gb|BM773686	4e-63
Meloidogyne incognita	ra85b02.y1	gb|BM773712	5e-63
Meloidogyne incognita	ra85f05.y1	gb|BM773761	5e-63
Meloidogyne incognita	ra86d03.y1	gb|BM773827	5e-63
Meloidogyne incognita	ra86g11.y1	gb|BM773868	1e-63
Meloidogyne incognita	ra87b02.y1	gb|BM773892	2e-63
Meloidogyne incognita	ra88b11.y1	gb|BM773988	5e-63
Meloidogyne incognita	ra88h01.y1	gb|BM774046	5e-63
Meloidogyne incognita	ra88h12.y1	gb|BM774056	5e-63
Meloidogyne incognita	ra91a06.y1	gb|BM774236	5e-63
Meloidogyne incognita	ra92c01.y1	gb|BM774335	5e-63
Meloidogyne incognita	ra92e09.y1	gb|BM774363	5e-63
Meloidogyne incognita	ra93f06.y1	gb|BM774450	1e-63
Meloidogyne incognita	ra94h05.y1	gb|BM774549	5e-63
Meloidogyne incognita	ra95g06.y1	gb|BM774622	3e-63
Meloidogyne incognita	ra95h06.y1	gb|BM774634	5e-63

Meloidogyne incognita	ra96g11.y1	gb|BM774715	5e-63
Meloidogyne incognita	ra97d11.y1	gb|BM774770	2e-63
Meloidogyne incognita	ra97f05.y1	gb|BM774785	5e-63
Meloidogyne incognita	ra97g12.y1	gb|BM774803	4e-63
Meloidogyne incognita	ra98c09.y1	gb|BM774842	5e-63
Meloidogyne incognita	ra98e05.y1	gb|BM774861	5e-63
Meloidogyne incognita	ra99f05.y1	gb|BM882343	5e-63
Meloidogyne incognita	rb01c12.y1	gb|BM882405	5e-63
Meloidogyne incognita	rb01h09.y1	gb|BM882460	5e-63
Meloidogyne incognita	rb02c02.y1	gb|BM882484	5e-63
Meloidogyne incognita	rb05e03.y1	gb|BM881054	5e-63
Meloidogyne incognita	rb06a03.y1	gb|BM881099	2e-63
Meloidogyne incognita	rb06d12.y1	gb|BM881142	5e-63
Meloidogyne incognita	rb07c08.y1	gb|BM881207	5e-63
Meloidogyne incognita	rb07g10.y1	gb|BM881252	5e-63
Meloidogyne incognita	rb08c10.y1	gb|BM881289	5e-63
Meloidogyne incognita	rb08h09.y1	gb|BM881343	5e-63
Meloidogyne incognita	rb09f08.y1	gb|BM881408	5e-63
Meloidogyne incognita	rb09g04.y1	gb|BM881416	5e-63
Meloidogyne incognita	rb11a06.y1	gb|BM881529	5e-63
Meloidogyne incognita	rb12h03.y1	gb|BM881691	5e-63
Meloidogyne incognita	rb12h10.y1	gb|BM881697	5e-63
Meloidogyne incognita	rb14g02.y1	gb|BM881850	2e-63
Meloidogyne incognita	rb14g04.y1	gb|BM881852	4e-63
Meloidogyne incognita	rb14g06.y1	gb|BM881854	1e-63
Meloidogyne incognita	rb15a03.y1	gb|BM881872	5e-63

Meloidogyne incognita	rb16b05.y1	gb|BM880600	5e-63
Meloidogyne incognita	rb16h07.y1	gb|BM880663	2e-63
Meloidogyne incognita	rb17b06.y1	gb|BM880685	5e-63
Meloidogyne incognita	rb18d06.y1	gb|BM880798	5e-63
Meloidogyne incognita	rb22e08.y1	gb|BM880457	5e-63
Meloidogyne incognita	rb24f01.y1	gb|BM881994	5e-63
Meloidogyne incognita	rb25f11.y1	gb|BM882090	3e-63
Meloidogyne incognita	rb25g03.y1	gb|BM882094	5e-63
Meloidogyne incognita	rb26d05.y1	gb|BM882147	2e-63
Meloidogyne incognita	rb26g11.y1	gb|BM882186	2e-63
Meloidogyne incognita	rb27a05.y1	gb|BM882203	5e-63
Meloidogyne incognita	rb29f01.y1	gb|BM882780	1e-63
Meloidogyne incognita	rb30a01.y1	gb|BM882816	5e-63
Meloidogyne incognita	rb30d12.y1	gb|BM882860	3e-63
Meloidogyne incognita	rb31h02.y1	gb|BM882986	5e-63
Meloidogyne javanica	rk43a07.y1	gb|BG735712	4e-63
Meloidogyne javanica	rk43e09.y1	gb|BG735752	2e-63
Meloidogyne javanica	rk43e12.y1	gb|BG735755	2e-63
Meloidogyne javanica	rk53e04.y1	gb|BG736231	2e-63
Meloidogyne javanica	rk53h02.y1	gb|BG736256	2e-63
Meloidogyne javanica	rk60b04.y1	gb|BG736624	2e-63
Meloidogyne javanica	rk62f12.y1	gb|BG736812	1e-63
Meloidogyne javanica	rk63c05.y1	gb|BG736846	2e-63
Meloidogyne javanica	rk65e08.y1	gb|BG737010	1e-63
Meloidogyne javanica	rk65g02.y1	gb|BG737025	3e-63
Meloidogyne javanica	rk66f07.y1	gb|BG737097	5e-63

Meloidogyne javanica	rk66g09.y1	gb|BG737108	2e-63
Meloidogyne javanica	rk66g10.y1	gb|BG737109	1e-63
Meloidogyne javanica	rk72b09.y1	gb|BI143215	3e-63
Meloidogyne javanica	rk81d09.y3	gb|BI745501	5e-63
Meloidogyne javanica	rk81d10.y3	gb|BI745502	2e-63
Meloidogyne javanica	rk81f12.y3	gb|BI745518	5e-63
Meloidogyne javanica	rk81g01.y3	gb|BI745519	5e-63
Meloidogyne javanica	rk89g05.y1	gb|BI744656	5e-63
Meloidogyne javanica	rk90d08.y1	gb|BI744549	5e-63
Meloidogyne javanica	rk90g10.y1	gb|BI744581	5e-63
Meloidogyne javanica	rk90g11.y1	gb|BI744582	2e-63
Meloidogyne javanica	rk91b12.y1	gb|BI744693	5e-63
Meloidogyne javanica	rk92a03.y1	gb|BI744754	3e-63
Meloidogyne javanica	rk97e03.y1	gb|BI745201	5e-63
Meloidogyne javanica	rk99c07.y1	gb|BI745347	5e-63
Meloidogyne javanica	rk99h08.y1	gb|BI745392	2e-63
Meloidogyne javanica	rl02d04.y1	gb|BI863247	2e-63
Meloidogyne javanica	rl05d03.y1	gb|BI863458	5e-63
Strongyloides ratti	ku14g06.y1	gb|BQ091242	2e-65
Strongyloides stercoralis	kp53h07.y1	gb|BE224025	5e-84
Strongyloides stercoralis	kq04b03.y1	gb|BG227238	2e-76
Strongyloides stercoralis	kq18e12.y1	gb|BG226203	4e-66

C. elegans gene: F25B4.6

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687438069	gb|BM592421	4e-44
Apis mellifera	BB170031B10F03.5	gb|BI505742	1e-45
Bombyx mori bra	AV400509	dbj|AV400509	4e-30
Necator americanus	Na_L3_09H09_SAC	gb|BU086573	4e-54
Strongyloides ratti	kt71f08.y1	gb|BI323469	1e-24

C. elegans gene: C45B2.7

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687494627	gb|BM626221	5e-16
Ancylostoma caninum	pb30d08.y1	gb|BM130388	3e-16
Ancylostoma caninum	pb44h12.y1	gb|BQ666635	9e-15
Ancylostoma caninum	pb09e11.y1	gb|BI744487	1e-13
Ancylostoma caninum	pb31h06.y1	gb|BQ125114	1e-13
Ancylostoma caninum	pb31h07.y1	gb|BQ125115	1e-13
Ancylostoma caninum	pb07d02.y1	gb|BI744318	5e-12
Ancylostoma caninum	pb30b09.y1	gb|BM130369	9e-12
Ancylostoma caninum	pb02b07.y1	gb|BF250603	4e-10
Ascaris suum	kh68b11.y1	gb|BM033843	1e-18
Ascaris suum	kk20b06.y1	gb|BQ096501	5e-13
Ascaris suum	kk27h06.y1	gb|BQ381130	4e-12
Brugia malayi	SWYD25CAU08D12SK	gb|AW257677	1e-14
Brugia malayi	kb34c04.y1	gb|BU917772	3e-11
Manduca sexta	EST292	gb|AI187503	3e-17
Meloidogyne arenaria	rm35b03.y1	gb|BI747039	1e-54
Meloidogyne javanica	rk99c03.y1	gb|BI745344	3e-31
Meloidogyne hapla	rc59a10.y1	gb|BM952341	4e-12
Meloidogyne arenaria	rm32d04.y1	gb|BI746830	1e-10
Onchocerca volvulus	SWOvAMCAQ10E05SK	gb|BE202282	1e-15
Ostertagia ostertagi	Oo_ad_02F04_LambdaGT11FO	gb|BG734000	4e-13
Parastrongyloides trichosuri	kx48f05.y1	gb|BI863807	3e-11
Strongyloides stercoralis	kq39f03.y1	gb|BE580303	3e-21

C. elegans gene: C37C3.3

Species	EST ID	Assession Number	E value
Aedes aegypti	EST	gb|BM144106	2e-11
Anopheles gambiae	17000687367709	gb|BM648797	1e-43
Anopheles gambiae	17000687384243	gb|BM590770	1e-41
Anopheles gambiae	17000687447857	gb|BM621866	1e-41
Ancylostoma caninum	pj99f09.y1	gb|CA033302	4e-11
Amblyomma variegatum	EST577974	gb|BM291440	6e-45
Bombyx mori	AU000259	dbj|AU000259	3e-50
Bombyx mori	AV401044	dbj|AV401044	2e-42
Bombyx mori	AU006392	dbj|AU006392	1e-40
Haemonchus contortus	Hc_d11_25E08_SKPL	gb|BF423278	4e-47
Ancylostoma caninum	pa46g09.y1	gb|AW735046	5e-27
Ancylostoma caninum	pb03g12.y1	gb|BF250735	8e-23
Zeldia punctata	rp11c10.y1	gb|AW773524	1e-46
Meloidogyne javanica	rk17h04.y1	gb|BE578050	2e-38
Meloidogyne javanica	rk52a07.y1	gb|BG736156	2e-24
Meloidogyne javanica	rk66e08.y1	gb|BG737087	1e-24
Necator americanus	Na_ad_01F02_SAC	gb|BG734490	2e-17
Pristionchus pacificus	rt01d05.y2	gb|BM812517	2e-58
Pristionchus pacificus	rt01d05.y1	gb|BM565711	3e-53
Pristionchus pacificus	rs26a01.y1	gb|AI988844	6e-18
Strongyloides stercoralis	kp41g07.y1	gb|BE030342	7e-51
Strongyloides stercoralis	kp18h06.y1	gb|AW588105	2e-39
Trichinella spiralis	pt34g08.y1	gb|BQ693409	1e-52
Trichinella spiralis	ps21a08.y4	gb|BG731987	1e-50

Trichinella spiralis	ps31d12.y1	gb|BG353562	3e-39
Trichinella spiralis	pt13a05.y1	gb|BQ693271	9e-35
Trichinella spiralis	ps31d12.y2	gb|BG438577	2e-29
Trichuris muris	Tm_ad_29E03_SKPL	gb|BM174441	2e-35
Trichuris muris	Tm_ad_08B07_SKPL	gb|BG577593	4e-35

C. elegans gene: F45G2.5

Species	EST ID	Assession Number	E value
Ostertagia ostertagi	ph79d04.y1	gb|BQ457535	6e-52

C. elegans gene: K08B4.1

Species	EST ID	Assession Number	E value
Brugia malayi	BSBmMFSZ22D12SK	gb|AW013739	2e-59
Brugia malayi	kb06e04.y1	gb|BM889162	7e-21
Heterodera glycines	ro82c01.y1	gb|BI748790	5e-21
Trichuris muris	Tm_ad_03F11_SKPL	gb|BF169284	3e-15

C. elegans gene: ZK970.4

Species	EST ID	Assession Number	E value
Caenorhabditis briggsae		gb|AC084593	1e-27
Manduca sexta		emb|X67130	3e-33
Anopheles gambiae		emb|Z69979	7e-31

C. elegans gene: H19M22.1

Species	EST ID	Assession Number	E value
Globodera rostochiensis	rr35f05.y2	gb|BM343207	2e-13
Ancylostoma caninum	pb02b10.y1	gb|BF250605	3e-17

C. elegans gene: ZK270.1

Species	EST ID	Assession Number	E value
Anopheles gambiae	17000687494627	gb|BM626221	1e-16
Ancylostoma caninum	pb30d08.y1	gb|BM130388	9e-98
Ancylostoma caninum	pb44h12.y1	gb|BQ666635	2e-90
Ancylostoma caninum	pb31h06.y1	gb|BQ125114	6e-86
Ancylostoma caninum	pb09e11.y1	gb|BI744487	5e-84
Ancylostoma caninum	pb31h07.y1	gb|BQ125115	3e-84
Ancylostoma caninum	pb07d02.y1	gb|BI744318	1e-79
Ancylostoma caninum	pb30b09.y1	gb|BM130369	1e-78
Ancylostoma caninum	pb29b03.y1	gb|BM130286	3e-75
Ancylostoma caninum	pb57c05.y1	gb|BQ667670	4e-52
Ancylostoma caninum	pb57d12.y1	gb|BQ667681	4e-52
Ancylostoma caninum	pb46a01.y1	gb|BQ666692	2e-50
Ancylostoma caninum	pb41h05.y1	gb|BQ666447	6e-11
Ascaris suum	kh68b11.y1	gb|BM033843	7e-20
Ascaris suum	kk20b06.y1	gb|BQ096501	6e-16
Ascaris suum	kk27h06.y1	gb|BQ381130	1e-14
Ascaris suum	kh95h02.y1	gb|BM285196	3e-12
Brugia malayi	SWYD25CAU08D12SK	gb|AW257677	3e-43
Brugia malayi	kb13a04.y1	gb|BU781174	5e-19
Globodera rostochiensis	rr59g08.y1	gb|BM344825	7e-18
Ancylostoma caninum	pb02b07.y1	gb|BF250603	1e-13
Litomosoides sigmodontis	JALsL3C008SAC	gb|AW152689	3e-16
Manduca sexta	EST292	gb|AI187503	1e-17

Meloidogyne hapla	rc61f09.y1	gb|BQ090105	2e-17
Meloidogyne hapla	rc59a10.y1	gb|BM952341	8e-16
Meloidogyne incognita	MD0882	gb|BE240858	6e-14
Meloidogyne arenaria	rm33a11.y1	gb|BI746878	2e-12
Meloidogyne hapla	rc55a06.y1	gb|BM952077	5e-12
Meloidogyne hapla	rc34h08.y1	gb|BM902339	6e-11
Meloidogyne javanica	rk57a05.y1	gb|BG736428	3e-11
Meloidogyne javanica	rk79a05.y1	gb|BI324434	6e-11
Necator americanus	Na_L3_09G07_SAC	gb|BU086563	3e-62
Necator americanus	Na_L3_36B10_SAC	gb|BU088351	2e-42
Necator americanus	Na_L3_12F04_SAC	gb|BU086791	1e-12
Onchocerca volvulus	SWOvAMCAQ10E05SK	gb|BE202282	2e-11
Ostertagia ostertagi	Oo_ad_02F04_LambdaGT11FO	gb|BG734000	2e-13
Parastrongyloides trichosuri	kx48f05.y1	gb|BI863807	4e-31
Parastrongyloides trichosuri	kx46c04.y1	gb|BI863606	8e-27
Pristionchus pacificus	rs10e10.r1	gb|AA193996	1e-62
Strongyloides stercoralis	kq31g12.y1	gb|BE579648	5e-45
Strongyloides stercoralis	kq39f03.y1	gb|BE580303	9e-15

For example, the *C. elegans* gene *mlt-12*, which corresponds to open reading frame W08F4.6, has exemplary orthologs in parasitic nematodes including BG310588 in *Onchocerca volvulus* (e^{-121}); BE758466 in *Brugia malayi* (e^{-104}); BG2271612 in *Strongyloides stercoralis* (e^{-84}); and BM3468116 in *Parastrongyloides trichosuri* (e^{-89}). The *C. elegans* gene *mlt-13*, which corresponds to open reading frame F09B12.1, has exemplary orthologs in parasitic nematodes including BG226227 in *Strongyloides stercoralis* ($9e^{-24}$) and BF169279 in *Trichuris muris* ($4e^{-11}$). The *C. elegans* gene *mlt-18*, which corresponds to open reading frame W01F3.3, has exemplary orthologs in parastic nematodes including BG893621 in *Strongyloides ratti* ($2e^{-20}$); BQ625515 in *Meloidogyne incognita* ($3e^{-25}$); and BI746672 in *Meloidogyne arenaria* ($6e^{-31}$). The *C. elegans* gene *mlt-14*, which corresponds to open reading frame C34G6.6, has exemplary orthologs in parastic nematodes including AA471404 in *Brugia malayi* ($2e^{-68}$); BE579677 in *Strongyloides stercoralis* ($2e^{-53}$); BI500192 in *Pristionchus pacificus* ($2e^{-69}$); BI782938 in *Ascaris suum* ($9e^{-52}$); BI073876 in *Strongyloides ratti* ($1e^{-41}$); and BF060055 in *Haemonchus contortus* ($4e^{-18}$). The *C. elegans* open reading frame ZK430.8 has an exemplary ortholog, AI723670, in *Brugia malayi* ($8e^{-40}$). The *C. elegans* gene *pan-1*, which corresponds to open reading frame M88.6 has exemplary orthologs in parastic nematodes including BI746256 in *Meloidogyne arenaria* ($3.^{00}e^{-15}$). The *C. elegans* gene *mlt-27*, which corresponds to open reading frame C42D8.5 has exemplary orthologs in parastic nematodes including BM882137 in *Parastrongyloides trichosuri* ($6e^{-33}$); BM277122 in *Trichuris muris* ($6e^{-15}$); BM880769 in *Meloidogyne incognita* ($3e^{-41}$);and BI501765 in *Meloidogyne arenaria*. The *C. elegans* gene *mlt-25* has exemplary orthologs in parasitic nematodes including BE581131 in *Strongyloides stercoralis* ($1e^{-34}$). *The C. elegans* open reading frame C23F12.1 has exemplary orthologs in parasitic nematodes including AI5399702 in *Onchocerca volvulus* (e^{-38}); BE5802318 in *Strongyloides stercoralis* (e^{-35}); BE2389166 *in Meloidogyne incognita* (e^{-17}); BI501765 in *Meloidogyne*

arenaria; BE581131 in *Strongyloides stercoralis* ($1e^{-34}$); AI5399702 in *Onchocerca volvulus* (e^{-38}); BE5802318 in *Strongyloides stercoralis* (e^{-35}); BE2389166 *in Meloidogyne incognita* (e^{-17}); BE580288 in *Strongyloides stercoralis;* AA161577 in *Brugia malayi* (e^{-39}); CAAC01000016 in *C. briggsae*; BI744615 in *Meloidogyne javanica* (4e-44); BG224680 *Strongyloides stercoralis* ($4e^{-44}$); AW114337 *Pristionchus pacificus* (e^{-41}), BM281377 in *Ascaris suum* ($2e^{-41}$); BU585500 in *Ascaris lumbricoides;* BG577863 in *Trichuris muris* (e^{-24}); BQ091075 in *Strongyloides ratti* ($6e^{-14}$); AW257707 in *Onchocerca volvulus*; BF014893 in *Strongyloides stercoralis* ($7e^{-35}$); BQ613344 in *Meloidogyne incognita* ($5e^{-47}$); CAAC01000088 in *C. Briggsae*, BG735742 in *Meloidogyne javanica* ($4e^{-14}$); CAAC01000028; AA110597 in *Brugia malayi* ($3e^{-56}$); BI863834 in *Parastrongyloides trichosuri* ($3e^{-69}$); AI987143 in *Pristionchus pacificus* ($3e^{-60}$); BI782814 in *Ascaris suum*; BI744849 in *Meloidogyne javanica*; and BG735807 in *Meloidogyne javanica* ($6e^{-38}$).

RNA interference

RNAi is a form of post-transcriptional gene silencing initiated by the introduction of double-stranded RNA (dsRNA) or antisense RNA. In *C. elegans* many expressed genes are subject to inactivation by RNAi (Fire et al., *Nature* 391:806-11, 1998; Fraser et al., *Nature* 408:325-30, 2000). RNAi may be accomplished by growing *C. elegans* on plates of *E. coli* expressing double stranded RNA. The nematodes feed on RNA-expressing bacteria, and this feeding is sufficient to cause the inactivation of specific target genes (Fraser et al., *Nature* 408:325-30, 2000; Kamath et al., *Genome Biol* 2, 2001). A double stranded RNA corresponding to one of the *mlt genes* described herein (e.g., one of those listed in Tables 1A, 1B, 4A-4D, and 7) is used to specifically silence *mlt* gene expression.

siRNA

Short twenty-one to twenty-five nucleotide double-stranded RNAs are effective at down-regulating gene expression in nematodes (Zamore et al., *Cell* 101: 25-33) and in mammalian tissue culture cell lines (Elbashir et al., *Nature* 411:494-498, 2001, hereby incorporated by reference). The further therapeutic effectiveness of this approach in mammals was demonstrated *in vivo* by McCaffrey et al. (*Nature* 418:38-39. 2002). The nucleic acid sequence of an Ecdysozoan gene ortholog can be used to design small interfering RNAs (siRNAs) that will inactivate *mlt* genes that have the specific 21 to 25 nucleotide RNA sequences used. siRNAs may be used, for example, as therapeutics to treat a parasitic nematode infection, as nematicides, or as insecticides.

Given the sequence of a *mlt* gene, siRNAs may be designed to inactivate that gene. For example, for a gene that consists of 2000 nucleotides, 1,978 different twenty-two nucleotide oligomers could be designed; this assumes that each oligomer has a two base pair 3' overhang, and that each siRNA is one nucleotide residue from the neighboring siRNA. To inactivate a gene, only a few of these twenty-two nucleotide oligomers would be needed; approximately one dozen siRNAs, spaced across the 2,000 nucleotide gene, would likely be sufficient to significantly reduce target gene activity in an Ecdysozoan. Such siRNAs, for example, could be administered directly to an affected tissue, or administered systemically. *C. elegans* is used to identify siRNAs that cause a Mlt phenotype or larval arrest.

siRNAs that target nucleic acid sequences conserved among *mlt* genes would be expected to inactivate the corresponding gene in any species having that sequence. Although the protein sequences of *mlt* genes are well conserved among widely divergent nematodes, for example, the nucleic acid sequences encoding them are not likely to exhibit the same level of conservation due to the degeneracy of the genetic code, which allows for wobble position

substitutions. Thus, many siRNAs are expected to inactivate mRNAs only in specific target species. An siRNA designed to target a divergent region of *O. volvulus mlt-12*, for example, would be unlikely to affect other species.

Druggable Targets

The genomic survey described herein has identified a number of enzymes with small molecule substrates that function in molting. The Ecdysozoan orthologs of these worm genes represent targets, in this case for the disruption of molting, which would traditionally be selected for development of small molecule drugs. The orthologs of *C. elegans* genes listed in Tables 1A, 1B, 4A-4D, and 7, for example, are novel candidates for the development of nematicides, insecticides, and therapeutics for the treatment of parasitic infections.

Proteases are a particularly promising target for anti-parasitic development since large protease inhibitor libraries presently exist (the legacy of the development of ACE inhibitors, more recently HIV protease inhibitors, and undoubtedly CED-3 like cysteine protease inhibitors) and may be screened to identify inhibitors. The chemical backbone of drugs designed against a class of proteases, such as a cysteine protease, may be used as a starting point for developing and designing drug targets against other members within that class of enzymes. In one embodiment, a candidate compound that inhibits a protease could be identified using standard methods to monitor protease biological activity, for example, substrate proteolysis. A decrease in substrate proteolysis in the presence of the candidate compound, as compared to substrate proteolysis in the absence of the candidate compound, identifies that candidate compound as useful in the methods of the invention. In fact, it is reasonable to expect the substrate of that protease to be present in the lists of *mlt* genes provided herein, for example, in Tables 1A, 1B, 4A-4D, and 7.

Protease/substrate pairs are identified by contacting recombinant proteases with recombinant candidate substrates and detecting substrate degradation or cleavage using an immunological assay, for example.

Isolation of Additional *mlt* Genes

Based on the nucleotide and amino acid sequences described herein, the isolation and identification of additional coding sequences of genes that function in molting is made possible using standard strategies and techniques that are well known in the art.

In one example, MLT polypeptides disclosed herein (e.g., those encoded by genes listed in Tables 1A, 1B, 4A-4D, and 7) are used to search a database, as described herein.

In another example, any organism that molts can serve as the nucleic acid source for the molecular cloning of such a gene, and these sequences are identified as ones encoding a protein exhibiting structures, properties, or activities associated with molt regulation disclosed herein (e.g., those listed in Tables 1A, 1B, 4A-4D, and 7).

In one particular example of such an isolation technique, any one of the nucleotide sequences described herein (e.g., those listed in Tables 1A, 1B, 4A-4D, and 7) may be used, together with conventional methods of nucleic acid hybridization screening. Such hybridization techniques and screening procedures are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci.*, USA 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York. In one particular example, all or part of a *mlt* nucleic acid sequences listed in Tables 1A, 1B, 4A-4D, and 7 may

be used as a probe to screen a recombinant DNA library for genes having sequence identity to a *mlt* gene. Hybridizing sequences are detected by plaque or colony hybridization according to standard methods.

Alternatively, using all or a portion of the nucleic acid sequence listed in Tables 1A, 1B, 4A-4D, and 7, one may readily design gene- or nucleic acid sequence-specific oligonucleotide probes, including degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either DNA strand and any appropriate portion of the nucleic acids, or nucleic acid sequences listed in Tables 1A, 1B, 4A-4D, and 7. General methods for designing and preparing such probes are provided, for example, in Ausubel et al. (supra), and Berger and Kimmel, (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York). These oligonucleotides are useful for *mlt* gene isolation or for the isolation of virtually any gene listed in Tables 1A, 1B, 4A-4D, and 7, either through their use as probes capable of hybridizing to a *mlt* gene, or as complementary sequences or as primers for various amplification techniques, for example, polymerase chain reaction (PCR) cloning strategies. If desired, a combination of different, detectably-labeled oligonucleotide probes may be used for the screening of a recombinant DNA library. Such libraries are prepared according to methods well known in the art, for example, as described in Ausubel et al. (supra), or they may be obtained from commercial sources.

As discussed above, sequence-specific oligonucleotides may also be used as primers in amplification cloning strategies, for example, using PCR. PCR methods are well known in the art and are described, for example, in *PCR Technology*, Erlich, ed., Stockton Press, London, 1989; *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc., New York, 1990; and Ausubel et al. (supra). Primers are optionally designed to allow cloning of the amplified product into a suitable vector, for example, by including appropriate restriction sites at the 5' and 3' ends of the amplified

fragment (as described herein). If desired, nucleotide sequences may be isolated using the PCR "RACE" technique, or Rapid Amplification of cDNA Ends (see, e.g., Innis et al. (supra)). By this method, oligonucleotide primers based on a desired sequence are oriented in the 3' and 5' directions and are used to generate overlapping PCR fragments. These overlapping 3'- and 5'-end RACE products are combined to produce an intact full-length cDNA. This method is described in Innis et al. (supra); and Frohman et al., (*Proc. Natl. Acad. Sci.* USA 85:8998, 1988).

Partial sequences, e.g., sequence tags, are also useful as hybridization probes for identifying full-length sequences, as well as for screening databases for identifying previously unidentified related virulence genes.

In general, the invention includes any nucleic acid sequence which may be isolated as described herein or which is readily isolated by homology screening or PCR amplification using any of the nucleic acid sequences disclosed herein (e.g., those listed in Tables 1A, 1B, 4A-4D, and 7).

It will be appreciated by those skilled in the art that, as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding *mlt* genes, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), or their variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7) under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide

sequences encoding *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), or their derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7) and their derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences that encode *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), or fragments thereof generated entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding any *mlt* gene (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to any *mlt* polynucleotide sequences (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35%

formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F. M. (1997) *Short Protocols in Molecular Biology*, John Wiley & Sons, New York N.Y., unit 7.7)

***In silico* Methods for the Isolation of Additional *mlt* Genes**

In addition to these experimental approaches for the identification of additional *mlt* genes, *mlt* genes are also identified *in silico* using routine methods known to one skilled in the art and described herein. Such methods include searching genomic and EST databases for orthologs of *C. elegans mlt* genes, for example, *mlt* genes shown in Tables 1A, 1B, 4A-4D, and 7. Thus, as new genome sequences become available for insect pests (e.g., the new mosquito genome sequence) or parasitic nematodes, the nucleic acid or protein sequence of any one of the *mlt* genes listed in Tables 1A, 1B, 4A-4D, and 7, as well as *mlt* genes identified according to the methods of the invention (e.g., those that are identified in an enhanced *mlt* screens using *C. elegans* mutants with an increased susceptibility to RNAi) may be used to identify *mlt* orthologs. New *mlt* genes, for example, those *mlt* genes that function in the nervous system may be used in blastn, blastp, and tblastn comparisons to seek orthologs in new and existing genome databases. Just as degenerate oligonucleotide probes can be used in PCR and hybridization experiments, virtual probes (e.g., those degenerate nucleic acid sequences encoding a MLT polypeptide) may be used to query genome and EST databases for orthologs. In this way, orthologs of additional *mlt* genes will emerge.

Significantly, genomes that lack one or more *mlt* orthologs will also be identified using these methods. Such analyses will allow for the identification of *mlt* genes that are conserved, for example, only in nematodes. This will allow the development of highly specific nematicides. The identification of *mlt*

genes that are conserved only among Ecdysozoans, and that are not present in vertebrates will allow the development of highly specific insecticides and nematicides unlikely to cause adverse side effects in vertebrates.

Polypeptide Expression

In general, MLT polypeptides of the invention may be produced by transformation of a suitable host cell with all or part of a *mlt* nucleic acid molecule (e.g., nucleic acids listed in Tables 1A, 1B, 4A-4D, and 7) or a fragment thereof in a suitable expression vehicle.

The MLT protein may be produced in a prokaryotic host, for example, *E. coli*, or in a eukaryotic host, for example, *Saccharomyces cerevisiae*, mammalian cells (for example, COS 1 or NIH 3T3 cells), or any of a number of plant cells or whole plant including, without limitation, algae, tree species, ornamental species, temperate fruit species, tropical fruit species, vegetable species, legume species, crucifer species, monocots, dicots, or in any plant of commercial or agricultural significance. Particular examples of suitable plant hosts include, but are not limited to, conifers, petunia, tomato, potato, pepper, tobacco, *Arabidopsis*, lettuce, sunflower, oilseed rape, flax, cotton, sugarbeet, celery, soybean, alfalfa, *Medicago*, lotus, *Vigna*, cucumber, carrot, eggplant, cauliflower, horseradish, morning glory, poplar, walnut, apple, grape, asparagus, cassava, rice, maize, millet, onion, barley, orchard grass, oat, rye, and wheat.

Such cells are available from a wide range of sources including the American Type Culture Collection (Rockland, MD); or from any of a number seed companies, for example, W. Atlee Burpee Seed Co. (Warminster, PA), Park Seed Co. (Greenwood, SC), Johnny Seed Co. (Albion, ME), or Northrup King Seeds (Harstville, SC). Descriptions and sources of useful host cells are also found in Vasil I.K., *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, II, III Laboratory Procedures and Their Applications Academic Press, New York, 1984; Dixon, R.A., *Plant Cell Culture-A Practical Approach*, IRL Press,

Oxford University, 1985; Green et al., *Plant Tissue and Cell Culture*, Academic Press, New York, 1987; and Gasser and Fraley, *Science* 244:1293, 1989.

One particular bacterial expression system for polypeptide production is the *E. coli* pET expression system (Novagen, Inc., Madison, WI). According to this expression system, DNA encoding a polypeptide is inserted into a pET vector in an orientation designed to allow expression. Since the gene encoding such a polypeptide is under the control of the T7 regulatory signals, expression of the polypeptide is achieved by inducing the expression of T7 RNA polymerase in the host cell. This is typically achieved using host strains which express T7 RNA polymerase in response to IPTG induction. Once produced, recombinant polypeptide is then isolated according to standard methods known in the art, for example, those described herein.

Another bacterial expression system for polypeptide production is the pGEX expression system (Pharmacia). This system employs a GST gene fusion system which is designed for high-level expression of genes or gene fragments as fusion proteins with rapid purification and recovery of functional gene products. The protein of interest is fused to the carboxyl terminus of the glutathione S-transferase protein from *Schistosoma japonicum* and is readily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B. Fusion proteins can be recovered under mild conditions by elution with glutathione. Cleavage of the glutathione S-transferase domain from the fusion protein is facilitated by the presence of recognition sites for site-specific proteases upstream of this domain. For example, proteins expressed in pGEX-2T plasmids may be cleaved with thrombin; those expressed in pGEX-3X may be cleaved with factor Xa.

Once the recombinant polypeptide of the invention is expressed, it is isolated, e.g., using affinity chromatography. In one example, an antibody (e.g., produced as described herein) raised against a polypeptide of the invention may be attached to a column and used to isolate the recombinant

polypeptide. Lysis and fractionation of polypeptide-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra).

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

Polypeptides of the invention, particularly short peptide fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). Also included in the invention are polypeptides which are modified in ways which do not abolish their biological activity (assayed, for example as described herein). Such changes may include certain mutations, deletions, insertions, or post-translational modifications, or may involve the inclusion of any of the polypeptides of the invention as one component of a larger fusion protein.

The invention further includes analogs of any naturally occurring polypeptide of the invention. Analogs can differ from the naturally occurring the polypeptide of the invention by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally occurring amino acid sequence of the invention. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or

following treatment with isolated modifying enzymes. Analogs can also differ from the naturally occurring polypeptides of the invention by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual* (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the invention also includes fragments of any one of the polypeptides of the invention. As used herein, the term "fragment," means at least 5, preferably at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of the invention can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events). The aforementioned general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein).

For eukaryotic expression, the method of transformation or transfection and the choice of vehicle for expression of the MLT polypeptide will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (*supra*); Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; Gelvin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990; Kindle, K., *Proc. Natl. Acad. Sci., U.S.A.* 87:1228, 1990; Potrykus, I., *Annu. Rev. Plant Physiol. Plant Mol. Biology* 42:205, 1991; and BioRad (Hercules, CA)

Technical Bulletin #1687 (Biolistic Particle Delivery Systems). Expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors: A Laboratory Manual* (P.H. Pouwels et al., 1985, Supp. 1987); Gasser and Fraley (*supra*); Clontech Molecular Biology Catalog (Catalog 1992/93 Tools for the Molecular Biologist, Palo Alto, CA); and the references cited above. Other expression constructs are described by Fraley et al. (U.S. Pat. No. 5,352,605).

Construction of Plant Transgenes

Transgenic plants containing a *mlt* transgene encoding a *mlt* polypeptide or containing a transgene encoding an RNA *mlt* nucleic acid inhibitor (e.g., dsRNA, siRNA, or antisense RNA) are useful for inhibiting molting in a Ecdysozoan contacting, feeding on, or parasitizing the plant. A transgenic plant, or population of such plants, expressing at least one *mlt* transgene (e.g., a MLT polypeptide or *mlt* nucleic acid inhibitor) would be expected to have increased resistance to Ecdysozoan (e.g., insect or nematode) damage or infestation. This is particularly desirable, given that Ecdysozoans can act as vectors for various plant diseases.

When designing an RNA *mlt* nucleic acid inhibitor for use in a transgenic plant, the specificity of the inhibitor must be considered. This is of particular importance when designing inhibitors that will induce plant immunity to Ecdysozoan (e.g., insect or nematode) infestation. In one particular example, the parasitic nematode, *Heterodera schachtii*, is a beet parasite that expresses a *mlt-14* ortholog. Expression of a *Heterodera schachtii*-specific RNA *mlt-14* nucleic acid inhibitor in transgenic beets would be expected to disrupt molting and inhibit only in *H. schactii*,or closely related sister species, but would not be expected to affect other nematodes, insects, or vertebrates. The methods of the invention provide for highly specific nematicides and insecticides that minimize the ecological consequences of

pesticide use. In most preferred embodiments, RNA *mlt* nucleic acid inhibitors target *mlt* genes conserved only in nematodes, and RNA *mlt* nucleic acid inhibitors are designed to target highly divergent regions of *mlt* genes.

For other applications an RNA *mlt* nucleic acid inhibitor that affects a wide range of E*cdysozoan* pests is useful. Such RNA *mlt* nucleic acid inhibitors are designed to target well conserved regions of a *mlt* gene. These RNA *mlt* nucleic acid inhibitors are particularly useful, for example, when crop damage is caused by a wide range of nematode or insect pests. As new genome sequences become available, the design of ever more selective RNA *mlt* nucleic acid inhibitors and chemical compounds that target particular *mlt* gene regions will become a simple matter of comparative genomics.

In the case of insecticide development, even though the discovery of insect *mlt* genes is predicated on the conservation of *mlt* protein sequences between insects and nematodes, it is expected that the nucleic acid sequence of the orthologous *mlt* genes may not be well conserved. Thus, dsRNA, for example, an RNA *mlt-14* nucleic acid inhibitor target just one particular pest. For other applications, it may be advantageous to target a particular region of a *mlt* gene that is well conserved among most insects. An RNA mlt nucleic acid inhibitor against a highly conserved region of a mlt gene would be useful, for example, in treating an area for a wide range of insect pests. As new genome sequences emerge, selection of compounds and nucleic acids that target particular *mlt* gene regions will become a simple matter of comparative genomics.

In one preferred embodiment, a *mlt* nucleic acid or RNA mlt nucleic acid inhibitor (e.g., double-stranded RNA, siRNA, or antisense RNA) is expressed by a stably-transfected plant cell line, a transiently-transfected plant cell line, or by a transgenic plant. A number of vectors suitable for stable or extrachromosomal transfection of plant cells or for the establishment of transgenic plants are available to the public; such vectors are described in

Pouwels et al. (*supra*), Weissbach and Weissbach (*supra*), and Gelvin et al. (*supra*). Methods for constructing such cell lines are described in, e.g., Weissbach and Weissbach (*supra*), and Gelvin et al. (*supra*).

Typically, plant expression vectors include (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (for example, one conferring inducible or constitutive, pathogen- or wound-induced, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Once the desired *mlt* nucleic acid sequence is obtained as described above, it may be manipulated in a variety of ways known in the art. For example, where the sequence involves non-coding flanking regions, the flanking regions may be subjected to mutagenesis.

A *mlt* DNA sequence of the invention may, if desired, be combined with other DNA sequences in a variety of ways. A *mlt* DNA sequence of the invention may be employed with all or part of the gene sequences normally associated with a *mlt* protein. In its component parts, a DNA sequence encoding an MLT protein is combined in a DNA construct having a transcription initiation control region capable of promoting transcription and translation in a host cell.

In general, the constructs will involve regulatory regions functional in plants which provide for modified production of MLT protein as discussed herein. The open reading frame coding for the MLT protein or functional fragment thereof will be joined at its 5' end to a transcription initiation regulatory region. Numerous transcription initiation regions are available which provide for constitutive or inducible regulation.

For applications where developmental, cell, tissue, hormonal, or environmental expression is desired, appropriate 5' upstream non-coding regions are obtained from other genes, for example, from genes regulated during meristem development, seed development, embryo development, or leaf development.

Regulatory transcript termination regions may also be provided in DNA constructs of this invention as well. Transcript termination regions may be provided by the DNA sequence encoding a MLT protein or any convenient transcription termination region derived from a different gene source. The transcript termination region will contain preferably at least 1-3 kb of sequence 3' to the structural gene from which the termination region is derived. Plant expression constructs having a *mlt* gene as the DNA sequence of interest for expression (in either the sense or antisense orientation) may be employed with a wide variety of plant life, particularly plant life involved in the production of storage reserves (for example, those involving carbon and nitrogen metabolism). Such genetically-engineered plants are useful for a variety of industrial and agricultural applications. Importantly, this invention is applicable to dicotyledons and monocotyledons, and will be readily applicable to any new or improved transformation or regeneration method.

The expression constructs include at least one promoter operably linked to at least one *mlt* gene (e.g., encoding a MLT polypeptide or RNA *mlt* nucleic acid inhibitor). An example of a useful plant promoter according to the invention is a caulimovirus promoter, for example, a cauliflower mosaic virus (CaMV) promoter. These promoters confer high levels of expression in most plant tissues, and the activity of these promoters is not dependent on virally encoded proteins. CaMV is a source for both the 35S and 19S promoters. Examples of plant expression constructs using these promoters are found in Fraley et al., U.S. Pat. No. 5,352,605. In most tissues of transgenic plants, the CaMV 35S promoter is a strong promoter (see, e.g., Odell et al., *Nature* 313:810, 1985). The CaMV promoter is also highly active in monocots (see,

e.g., Dekeyser et al., *Plant Cell* 2:591, 1990; Terada and Shimamoto, *Mol. Gen. Genet.* 220:389, 1990). Moreover, activity of this promoter can be further increased (i.e., between 2-10 fold) by duplication of the CaMV 35S promoter (see e.g., Kay et al., *Science* 236:1299, 1987; Ow et al., *Proc. Natl. Acad. Sci., U.S.A.* 84:4870, 1987; and Fang et al., *Plant Cell* 1:141, 1989, and McPherson and Kay, U.S. Pat. No. 5,378,142).

Other useful plant promoters include, without limitation, the nopaline synthase (NOS) promoter (An et al., *Plant Physiol.* 88:547, 1988 and Rodgers and Fraley, U.S. Pat. No. 5,034,322), the octopine synthase promoter (Fromm et al., *Plant Cell* 1:977, 1989), figwort mosiac virus (FMV) promoter (Rodgers, U.S. Pat. No. 5,378,619), and the rice actin promoter (Wu and McElroy, W091/09948).

Exemplary monocot promoters include, without limitation, commelina yellow mottle virus promoter, sugar cane badna virus promoter, rice tungro bacilliform virus promoter, maize streak virus element, and wheat dwarf virus promoter.

For certain applications, it may be desirable to produce the MLT gene product in an appropriate tissue, at an appropriate level, or at an appropriate developmental time. For this purpose, there are an assortment of gene promoters, each with its own distinct characteristics embodied in its regulatory sequences, shown to be regulated in response to inducible signals such as the environment, hormones, and/or developmental cues. These include, without limitation, gene promoters that are responsible for heat-regulated gene expression (see, e.g., Callis et al., *Plant Physiol.* 88:965, 1988; Takahashi and Komeda, *Mol. Gen. Genet.* 219:365, 1989; and Takahashi et al. *Plant J.* 2:751, 1992), light-regulated gene expression (e.g., the pea *rbcS-3A* described by Kuhlemeier et al., *Plant Cell* 1:471, 1989; the maize *rbcS* promoter described by Schäffner and Sheen, *Plant Cell* 3:997, 1991; the chlorophyll a/b-binding protein gene found in pea described by Simpson et al., *EMBO J.* 4:2723, 1985; the Arabssu promoter; or the rice rbs promoter), hormone-regulated gene

expression (for example, the abscisic acid (ABA) responsive sequences from the *Em* gene of wheat described by Marcotte et al., *Plant Cell* 1:969, 1989; the ABA-inducible HVA1 and HVA22, and rd29A promoters described for barley and *Arabidopsis* by Straub et al., *Plant Cell* 6:617, 1994 and Shen et al., *Plant Cell* 7:295, 1995; and wound-induced gene expression (for example, of *wunI* described by Siebertz et al., *Plant Cell* 1:961, 1989), organ-specific gene expression (for example, of the tuber-specific storage protein gene described by Roshal et al., *EMBO J.* 6:1155, 1987; the 23-kDa zein gene from maize described by Schernthaner et al., *EMBO J.* 7:1249, 1988; or the French bean ß-phaseolin gene described by Bustos et al., *Plant Cell* 1:839, 1989), or pathogen-inducible promoters (for example, PR-1, prp-1, or -1,3 glucanase promoters, the fungal-inducible wirla promoter of wheat, and the nematode-inducible promoters, TobRB7-5A and Hmg-1, of tobacco and parsley, respectively).

Plant expression vectors may also optionally include RNA processing signals, e.g., introns, which have been shown to be important for efficient RNA synthesis and accumulation (Callis et al., *Genes and Dev.* 1:1183, 1987). The location of the RNA splice sequences can dramatically influence the level of transgene expression in plants. In view of this fact, an intron may be positioned upstream or downstream of an MLT polypeptide-encoding sequence in the transgene to modulate levels of gene expression.

In addition to the aforementioned 5' regulatory control sequences, the expression vectors may also include regulatory control regions which are generally present in the 3' regions of plant genes (Thornburg et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:744, 1987; An et al., *Plant Cell* 1:115, 1989). For example, the 3' terminator region may be included in the expression vector to increase stability of the mRNA. One such terminator region may be derived from the PI-II terminator region of potato. In addition, other commonly used terminators are derived from the octopine or nopaline synthase signals.

The plant expression vector also typically contains a dominant selectable marker gene used to identify those cells that have become transformed. Useful selectable genes for plant systems include genes encoding antibiotic resistance genes, for example, those encoding resistance to hygromycin, kanamycin, bleomycin, G418, streptomycin, or spectinomycin. Genes required for photosynthesis may also be used as selectable markers in photosynthetic-deficient strains. Finally, genes encoding herbicide resistance may be used as selectable markers; useful herbicide resistance genes include the *bar* gene encoding the enzyme phosphinothricin acetyltransferase and conferring resistance to the broad spectrum herbicide Basta® (Frankfurt, Germany).

Efficient use of selectable markers is facilitated by a determination of the susceptibility of a plant cell to a particular selectable agent and a determination of the concentration of this agent which effectively kills most, if not all, of the transformed cells. Some useful concentrations of antibiotics for tobacco transformation include, e.g., 75-100 μg/mL (kanamycin), 20-50 μg/mL (hygromycin), or 5-10 μg/mL (bleomycin). A useful strategy for selection of transformants for herbicide resistance is described, e.g., by Vasil et al., *supra*.

In addition, if desired, the plant expression construct may contain a modified or fully-synthetic structural *mlt* coding sequence that has been changed to enhance the performance of the gene in plants. Methods for constructing such a modified or synthetic gene are described in Fischoff and Perlak, U.S. Pat. No. 5,500,365.

It should be readily apparent to one skilled in the art of molecular biology, especially in the field of plant molecular biology, that the level of gene expression is dependent, not only on the combination of promoters, RNA processing signals, and terminator elements, but also on how these elements are used to increase the levels of selectable marker gene expression.

Plant Transformation

Upon construction of the plant expression vector, several standard methods are available for introduction of the vector into a plant host, thereby generating a transgenic plant. These methods include (1) Agrobacterium-mediated transformation (*A. tumefaciens* or *A. rhizogenes*) (see, e.g., Lichtenstein and Fuller In: *Genetic Engineering*, vol 6, PWJ Rigby, ed, London, Academic Press, 1987; and Lichtenstein, C.P., and Draper, J,. In: *DNA Cloning*, Vol II, D.M. Glover, ed, Oxford, IRI Press, 1985)), (2) the particle delivery system (see, e.g., Gordon-Kamm et al., *Plant Cell* 2:603 (1990); or BioRad Technical Bulletin 1687, *supra*), (3) microinjection protocols (see, e.g., Green et al., *supra*), (4) polyethylene glycol (PEG) procedures (see, e.g., Draper et al., *Plant Cell Physiol.* 23:451, 1982; or e.g., Zhang and Wu, *Theor. Appl. Genet.* 76:835, 1988), (5) liposome-mediated DNA uptake (see, e.g., Freeman et al., *Plant Cell Physiol.* 25:1353, 1984), (6) electroporation protocols (see, e.g., Gelvin et al., *supra*; Dekeyser et al., *supra*; Fromm et al., *Nature* 319:791, 1986; Sheen *Plant Cell* 2:1027, 1990; or Jang and Sheen *Plant Cell* 6:1665, 1994), and (7) the vortexing method (see, e.g., Kindle *supra*). The method of transformation is not critical to the invention. Any method which provides for efficient transformation may be employed. As newer methods are available to transform crops or other host cells, they may be directly applied. Suitable plants for use in the practice of the invention include, but are not limited to, sugar cane, wheat, rice, maize, sugar beet, potato, barley, manioc, sweet potato, soybean, sorghum, cassava, banana, grape, oats, tomato, millet, coconut, orange, rye, cabbage, apple, watermelon, canola, cotton, carrot, garlic, onion, pepper, strawberry, yam, peanut, onion, bean, pea, mango, citrus plants, walnuts, and sunflower.

The following is an example outlining one particular technique, an *Agrobacterium*-mediated plant transformation. By this technique, the general process for manipulating genes to be transferred into the genome of plant cells is carried out in two phases. First, cloning and DNA modification steps are

carried out in *E. coli*, and the plasmid containing the gene construct of interest is transferred by conjugation or electroporation into *Agrobacterium*. Second, the resulting *Agrobacterium* strain is used to transform plant cells. Thus, for the generalized plant expression vector, the plasmid contains an origin of replication that allows it to replicate in *Agrobacterium* and a high copy number origin of replication functional in *E. coli*. This permits facile production and testing of transgenes in *E. coli* prior to transfer to *Agrobacterium* for subsequent introduction into plants. Resistance genes can be carried on the vector, one for selection in bacteria, for example, streptomycin, and another that will function in plants, for example, a gene encoding kanamycin resistance or herbicide resistance. Also present on the vector are restriction endonuclease sites for the addition of one or more transgenes and directional T-DNA border sequences which, when recognized by the transfer functions of *Agrobacterium*, delimit the DNA region that will be transferred to the plant.

In another example, plant cells may be transformed by shooting into the cell tungsten microprojectiles on which cloned DNA is precipitated. In the Biolistic Apparatus (Bio-Rad) used for the shooting, a gunpowder charge (22 caliber Power Piston Tool Charge) or an air-driven blast drives a plastic macroprojectile through a gun barrel. An aliquot of a suspension of tungsten particles on which DNA has been precipitated is placed on the front of the plastic macroprojectile. The latter is fired at an acrylic stopping plate that has a hole through it that is too small for the macroprojectile to pass through. As a result, the plastic macroprojectile smashes against the stopping plate, and the tungsten microprojectiles continue toward their target through the hole in the plate. For the instant invention the target can be any plant cell, tissue, seed, or embryo. The DNA introduced into the cell on the microprojectiles becomes integrated into either the nucleus or the chloroplast.

In general, transfer and expression of transgenes in plant cells are now routine for one skilled in the art, and have become major tools to carry out gene expression studies in plants and to produce improved plant varieties of agricultural or commercial interest.

Transgenic Plant Regeneration

Plant cells transformed with a plant expression vector can be regenerated, for example, from single cells, callus tissue, or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues, and organs from almost any plant can be successfully cultured to regenerate an entire plant; such techniques are described, e.g., in Vasil *supra*; Green et al., *supra*; Weissbach and Weissbach, *supra*; and Gelvin et al., *supra*.

In one particular example, a cloned MLT polypeptide expression construct under the control of the 35S CaMV promoter and the nopaline synthase terminator and carrying a selectable marker (for example, kanamycin resistance) is transformed into *Agrobacterium*. Transformation of leaf discs, with vector-containing *Agrobacterium* is carried out as described by Horsch et al. (*Science* 227:1229, 1985). Putative transformants are selected after a few weeks (for example, 3 to 5 weeks) on plant tissue culture media containing kanamycin (e.g. 100 μg/mL). Kanamycin-resistant shoots are then placed on plant tissue culture media without hormones for root initiation. Kanamycin-resistant plants are then selected for greenhouse growth. If desired, seeds from self-fertilized transgenic plants can then be sowed in a soil-less medium and grown in a greenhouse. Kanamycin-resistant progeny are selected by sowing surfaced sterilized seeds on hormone-free kanamycin-containing media. Analysis for the integration of the transgene is accomplished by standard techniques (see, for example, Ausubel et al. *supra*; Gelvin et al. *supra*).

Transgenic plants expressing the selectable marker are then screened for transmission of the transgene DNA by standard immunoblot and DNA detection techniques. Each positive transgenic plant and its transgenic progeny are unique in comparison to other transgenic plants established with the same transgene. Integration of the transgene DNA into the plant genomic DNA is in most cases random, and the site of integration can profoundly affect the levels and the tissue and developmental patterns of transgene expression. Consequently, a number of transgenic lines are usually screened for each transgene to identify and select plants with the most appropriate expression profiles.

Transgenic lines are evaluated for levels of transgene expression. Expression at the RNA level is determined initially to identify and quantitate expression-positive plants. Standard techniques for RNA analysis are employed for transgenic plants expressing RNA *mlt* nucleic acid inhibitors and *mlt* nucleic acids encoding a MLT polypeptide. Such techniques include PCR amplification assays using oligonucleotide primers designed to amplify only transgene RNA templates and solution hybridization assays using transgene-specific probes (see, e.g., Ausubel et al., *supra*). Those RNA-positive plants that encode a MLT protein are then analyzed for protein expression by Western immunoblot analysis using MLT specific antibodies (see, e.g., Ausubel et al., *supra*). In addition, *in situ* hybridization and immunocytochemistry according to standard protocols can be done using transgene-specific nucleotide probes and antibodies, respectively, to localize sites of expression within transgenic tissue.

Ectopic expression of one or more *mlt* genes or RNA *mlt* nucleic acid inhibitors is useful for the production of transgenic plants that disrupt molting in an Ecdysozoan (e.g., an insect or nematode) and have an increased level of resistance to insect or nematode infestation.

Transgenic Plants Expressing a *mlt* Transgene Disrupt Molting in an Insect or a Nematode

As discussed above, plasmid constructs designed for the expression of *mlt* nucleic acids or RNA *mlt* nucleic acid inhibitors (e.g., double-stranded RNA, siRNA, or antisense RNA) are useful, for example, for inhibiting molting in an Ecdysozoan (e.g., a parasitic insect or nematode) in contact with a transgenic plant transformed with at least one *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor. *mlt* nucleic acids that are isolated from an Ecdysozoan may be engineered for expression in a plant. The *mlt* nucleic acid may be expressed in its entirety, a portion of the *mlt* nucleic acid may be expressed, or an RNA *mlt* nucleic acid inhibitor comprising a *mlt* nucleic acid, or comprising the complementary strand of a *mlt* nucleic acid, may be expressed. The portion (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 95%) of the full length nucleic acid may be selected to maximize specificity and minimize the effect of the nucleic acid expression on, for example, beneficial insects or nematodes. To disrupt molting in an Ecdysozoan, it is important to express an MLT protein or RNA *mlt* nucleic acid inhibitor at an effective level. Evaluation of the level of insect or nematode protection conferred to a plant by ectopic expression of a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor is determined according to conventional methods and assays.

In one embodiment, constitutive ectopic expression of a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor is generated by transforming a plant with a plant expression vector containing a nucleic acid sequence encoding an MLT polypeptide or RNA *mlt* nucleic acid inhibitor (e.g., double stranded RNA, antisense RNA, or siRNA). This expression vector is then used to transform a plant according to standard methods known to the skilled artisan and described in Fischhoff et al. (U.S. Patent 5,500,365).

To assess resistance to nematodes or insects, transformed plants and appropriate control plants not expressing a transgene are grown to maturity, and a harmful insect or nematode is introduced to the plant under controlled

conditions (for example, standard levels of temperature, humidity, and/or soil conditions). After a period of incubation sufficient to allow the growth and reproduction of a harmful insect or nematode on a control plant, nematodes or insects on transgenic and control plants are evaluated for their level of growth, viability, or reproduction according to conventional experimental methods. In one embodiment, the number of insects or nematodes and their progeny is recorded every twenty-four hours for seven days, fourteen days, twenty-one days, or twenty-eight days after inoculation. From these data, the effectiveness of transgene expression is determined. Transformed plants expressing a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor that inhibits the growth, viability, or reproduction of a harmful insect or nematode relative to control plants are taken as being useful in the invention.

In another embodiment, plant damage in response to infestation with a harmful insect or parasitic nematode is evaluated according to standard methods. The level of insect or nematode damage in a plant expressing a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor relative to a control plant not transformed with a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor are compared. Transformed plants expressing a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor that protects the plant from insect or nematode infestation, relative to a control plant not expressing a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor, are taken as being useful in the invention.

Plants expressing a *mlt* nucleic acid or RNA *mlt* nucleic acid inhibitor (e.g., a *mlt* double-stranded RNA, antisense RNA or siRNA) are less vulnerable to insects, nematodes, and pest-transmitted diseases. The invention further provides for increased production efficiency, as well as for improvements in quality and yield of crop plants and ornamentals. Thus, the invention contributes to the production of high quality and high yield agricultural products, for example, fruits, ornamentals, vegetables, cereals and field crops having reduced spots, blemishes, and blotches that are caused by insects or nematodes; agricultural products with increased shelf-life and

reduced handling costs; and high quality and yield crops for agricultural (for example, cereal and field crops), industrial (for example, oilseeds), and commercial (for example, fiber crops) purposes. Furthermore, because the invention reduces the necessity for chemical protection against plant pathogens, the invention benefits the environment where the crops are grown. Genetically-improved seeds and other plant products that are produced using plants expressing the nucleic acids described herein also render farming possible in areas previously unsuitable for agricultural production.

Production of Transgenic Domestic Mammals

Domesticated mammals (such as a cow, sheep, goat, pig, horse, dog, or cat) expressing a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA) are useful for inhibiting molting in an Ecdysozoan contacting (e.g., feeding on or parasitizing) the mammal. Such transgenic mammals will be resistant to Ecdysozoan parasites and will be useful in controlling insect or parasite infestation, or the spread of diseases transmitted by Ecdysozoan vectors. Methods for generating a transgenic mammal are known to the skilled artisan, and are described, for example, in WO 02/51997 and WO 02/070648. Transgenic mammals may be produced using standard methods for nuclear transfer, embryonic activation, embryo culture, and embryo transfer. Traditional methods for generating such mammals are described by Cibelli *et al.* (Science 1998: 280:1256-1258).

Production of Transgenic Ecdysozoans

Some human parasites spend a part of their life cycle parasitizing an insect host. Methods of the invention are useful in controlling such parasites. Transgenic insect hosts (e.g., Drosophila) expressing a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA) are useful for inhibiting molting in an Ecdysozoan (e.g., nematode)

parasitizing the insect host. Such transgenic insects will be useful in controlling parasite infestation, or the spread of diseases transmitted by Ecdysozoan vectors.

In one embodiment, an insect (e.g., a black fly) is transformed with an RNA *mlt* nucleic acid inhibitor. Expression of the RNA *mlt* nucleic acid inhibitor kills parasitic nematode larvae (e.g., *Onchocerca volvulus*) within the insect host.

In another embodiment, transgenic Ecdysozoans (e.g., insects or nematodes) expressing a *mlt* nucleic acid or an RNA *mlt* nucleic acid inhibitor (e.g., double-stranded RNA, antisense RNA, or siRNA) are useful for inhibiting molting in an Ecdysozoan contacting (e.g., breeding with) the insect. A transgenic Ecdysozoan is bred to a naturally occurring Ecdysozoan to inhibit molting in the progeny and control an Ecdysozoan pest population. Methods for generating transgenic insects and nematodes are known to the skilled artisan, and are described, for example, by Kassis et al., (*PNAS* 89:1919-1923, 1992) and Chalfie et al., (*Science* 263:802-5, 1994).

Antibodies

The polypeptides disclosed herein or variants thereof or cells expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides. "Antibodies" as used herein include monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunolglobulin expression library.

To generate antibodies, a coding sequence for a polypeptide of the invention may be expressed as a C-terminal fusion with glutathione S-transferase (GST) (Smith et al., *Gene* 67:31, 1988). The fusion protein is purified on glutathione-Sepharose beads, eluted with glutathione, cleaved with thrombin (at the engineered cleavage site), and purified to the degree necessary for immunization of rabbits. Primary immunizations are carried out with

Freund's complete adjuvant and subsequent immunizations with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved protein fragment of the GST fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled protein. Antiserum specificity is determined using a panel of unrelated GST proteins.

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique immunogenic regions of a polypeptide of the invention may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity tested in ELISA and Western blots using peptide conjugates, and by Western blot and immunoprecipitation using the polypeptide expressed as a GST fusion protein.

Alternatively, monoclonal antibodies which specifically bind any one of the polypeptides of the invention are prepared according to standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, NY, 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specific recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize the polypeptide of the invention are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay. Alternatively monoclonal antibodies may be prepared using the polypeptide of the invention described above and a phage display library (Vaughan et al., *Nature Biotech* 14:309, 1996).

Preferably, antibodies of the invention are produced using fragments of the polypeptides disclosed herein which lie outside generally conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged

residues. In one specific example, such fragments are generated by standard techniques of PCR and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). To attempt to minimize the potential problems of low affinity or specificity of antisera, two or three such fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in a series, preferably including at least three booster injections.

Diagnostics

In another embodiment, antibodies which specifically bind any of the polypeptides described herein may be used for the diagnosis of a parasite infection, or a parasite-related disease. A variety of protocols for measuring such polypeptides, including immunological methods (such as ELISAs and RIAs) and FACS, are known in the art and provide a basis for diagnosing a parasite infection or a parasite-related disease.

In another aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including *mlt* genomic sequences, *mlt* open reading frames, or closely related molecules may be used to identify nucleic acid sequences which encode a MLT gene product. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7), allelic variants, or related sequences. Hybridization techniques may be used to identify mutations in *mlt* genes or may be used to monitor expression levels of these genes (for example, by Northern analysis, (Ausubel et al., *supra*).

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan et al., U.S. Pat. No. 5,474,796; Schena et al., *Proc. Natl. Acad. Sci.* 93:10614, 1996; Baldeschweiler et al., PCT application WO95/251116, 1995; Shalon, D. et al., PCT application WO95/35505, 1995; Heller et al., *Proc. Natl. Acad. Sci.* 94:2150, 1997; and Heller et al., U.S. Pat. No. 5,605,662.)

Screening Assays

As discussed above, the identified *mlt* genes (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7) function in Ecdysozoan molting. Based on this discovery, screening assays were developed to identify compounds that inhibit the action of a MLT polypeptide or the expression of a *mlt* nucleic acid sequence. The method of screening may involve high-throughput techniques. In addition, these screening techniques may be carried out in cultured cells or in animals (such as nematodes).

Any number of methods are available for carrying out such screening assays. In one working example, candidate compounds are added at varying concentrations to the culture medium of cultured cells or nematodes expressing one of the nucleic acid sequences of the invention. Gene expression is then measured, for example, by standard Northern blot analysis (Ausubel et al., supra) or RT-PCR, using any appropriate fragment prepared from the nucleic acid molecule as a hybridization probe. The level of gene expression in the presence of the candidate compound is compared to the level measured in a control culture medium lacking the candidate molecule. A compound which

promotes a decrease in the expression of a *mlt* gene (e.g., a gene listed in Tables 1A, 1B, 4A-4D, and 7) or functional equivalent is considered useful in the invention; such a molecule may be used, for example, as a nematicide, insecticide, or therapeutic to treat a parasitic-nematode infection. Such cultured cells include nematode cells (for example, *C. elegans* cells), mammalian, or insect cells.

In another working example, the effect of candidate compounds may be measured at the level of polypeptide production using the same general approach and standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific for a MLT polypeptide encoded by a *mlt* gene (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7). For example, immunoassays may be used to detect or monitor the expression of at least one of the polypeptides of the invention in an organism. Polyclonal or monoclonal antibodies (produced as described above) which are capable of binding to such a polypeptide may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to measure the level of the polypeptide. A compound that promotes a decrease in the expression of the polypeptide is considered particularly useful. Again, such a molecule may be used, for example, as a nematicide, insecticide, or therapeutic to delay, ameliorate, or treat a parasitic nematode infection.

In yet another working example, candidate compounds may be screened for those which specifically bind to and antagonize a MLT polypeptide encoded by a *mlt* gene (e.g., genes listed in Tables 1A, 1B, 4A-4D, and 7). The efficacy of such a candidate compound is dependent upon its ability to interact with a MLT polypeptide or a functional equivalent thereof. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described in Ausubel et al., supra). For example, a candidate compound may be tested *in vitro* for interaction and binding with a polypeptide of the invention and its ability to modulate molting may be assayed by any standard assay (e.g., those described herein).

In one particular working example, a candidate compound that binds to a MLT polypeptide, i.e., a polypeptide encoded by a *mlt* gene (e.g., a gene listed in Tables 1A, 1B, 4A-4D, and 7) may be identified using a chromatography-based technique. For example, a recombinant polypeptide of the invention may be purified by standard techniques from cells engineered to express the polypeptide (e.g., those described above) and may be immobilized on a column. A solution of candidate compounds is then passed through the column, and a compound specific for the MLT polypeptide is identified on the basis of its ability to bind to the MLT polypeptide and be immobilized on the column. To isolate the compound, the column is washed to remove non-specifically bound molecules, and the compound of interest is then released from the column and collected. Compounds isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography). In addition, these candidate compounds may be tested for their ability to disrupt molting (e.g., as described herein). Compounds isolated by this approach may also be used, for example, as nematicides, insecticides, or therapeutics to treat a parasitic nematode infection. Compounds which are identified as binding to a MLT polypeptide with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention. Alternatively, any *in vivo* protein interaction detection system, for example, any two-hybrid assay may be utilized.

Potential antagonists include organic molecules, peptides, peptide mimetics, polypeptides, nucleic acids, and antibodies that bind to a nucleic acid sequence or polypeptide of the invention (e.g., MLT polypeptide) and thereby decrease its activity. Potential antagonists also include small molecules that bind to and occupy the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented.

Each of the DNA sequences provided herein may also be used in the discovery and development of a nematicide, insecticide, or therapeutic compound for the treatment of parasitic nematode infection. The encoded protein, upon expression, can be used as a target for the screening of molt-disrupting drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest. Such sequences may be isolated by standard techniques (Ausubel et al., *supra*).

The antagonists of the invention may be employed, for instance, as nematicides, insecticides, or therapeutics for the treatment of a parasitic nematode infection.

Optionally, compounds identified in any of the above-described assays may be confirmed as useful in a *C. elegans* molting assay.

Small molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

Test Compounds and Extracts

In general, compounds capable of disrupting molting are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Compounds used in screens may include known compounds (for example, known therapeutics used for other diseases or disorders). Alternatively, virtually any number of unknown chemical extracts or compounds can be screened using the methods described herein. Examples

of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their molt-disrupting activity should be employed whenever possible.

When a crude extract is found to have a molt-disrupting activity, or a binding activity, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having molt-disrupting activity. Methods of fractionation and purification of such heterogenous extracts are known in the art. If desired, compounds shown

to be useful as insecticides, nematicides, or therapeutics for the treatment of a parasitic nematode infection are chemically modified according to methods known in the art.

Pharmaceutical Therapeutics

The invention provides a simple means for identifying compounds (including peptides, small molecule inhibitors, and mimetics) capable of acting as therapeutics for the treatment of a parasitic nematode infection. Accordingly, a chemical entity discovered to have medicinal value using the methods described herein is useful as a drug or as information for structural modification of existing insecticides or nematicides compounds, e.g., by rational drug design. Such methods are useful for screening compounds having an effect on a variety of conditions involving parasitic nematode infections in animals, for example, mammals, including humans and domestic animals (e.g., virtually any bovine, canine, caprine, feline, ovine, or porcine species). Parasitic nematodes that infect animals include, but are not limited to, any ascarid, filarid, or rhabditid (e.g., *Dioctophymatida, Dioctophyme renale, Eustrongylides tubifex, Trichurida, Capillaria hepatica, Capillaria philippinensis, Trichinella spiralis, Trichuris muris, Trichuris, Trichuris trichiura, Trichuris vulpis. Ancylostoma, Ancylostoma caninum, Ancylostoma duodenal, Ancylostoma braziliense, Necator, Necator americanus, Placoconus, Angiostrongylus cantonensis, Cooperia, Haemonchus, Nematodirus, Obeliscoides cuniculi, Ostertagia, Trichostongylus, Ascaris, Ascaris lumbricoides, Ascaris suum, Toxocara canis, Baylisascaris procyonis,Anisakis, Oxyurida, Enterobius vermicularis, Cosmocerella, Onchocercidae, Brugia malayi, Dirofilaria, Dirofilaria immitis, Loa loa, Onchocerca volvulus, Wuchereria bancrofti, Spinitectus, Camallanus, Camallanus oxycephalus, Dracunculus, Dracunculus medinensis, Philometra cylindracea, Heterorhabditis bacteriophora, Parastrongyloides trichosuri, Pristionchus pacificus, Steinernema, Strongyloides stercoralis,* or *Strongyloides ratti).*

For therapeutic uses, the compositions or agents identified using the methods disclosed herein may be administered systemically, for example, formulated in a pharmaceutically-acceptable buffer such as physiological saline. Preferable routes of administration include, for example, subcutaneous, intravenous, interperitoneally, intramuscular, or intradermal injections that provide continuous, sustained levels of the drug in the patient. Treatment of human patients or other animals will be carried out using a therapeutically effective amount of a parasite inhibitory agent in a physiologically-acceptable carrier. Suitable carriers and their formulation are described, for example, in Remington's Pharmaceutical Sciences by E.W. Martin. The amount of the nematicide agent to be administered varies depending upon the manner of administration, the age and body weight of the patient, and with the type of parasite the extensiveness of the infection. Generally, amounts will be in the range of those used for other agents used in the treatment of other diseases associated with parasite infection, although in certain instances lower amounts will be needed because of the increased specificity of the compound. In some applications, higher concentrations of the agent may be used given that the compound is highly specific to nematodes, and is therefore less likely to have adverse side effects in humans. A compound is administered at a dosage that induces larval arrest, disrupts nematode molting, or inhibits nematode viability.

Formulation of Pharmaceutical Compositions

The administration of an anti-parasitic compound may be by any suitable means that results in a concentration of the anti-parasitic that, combined with other components, is anti-parasitic upon reaching the parasite target. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, intramuscularly, or intraperitoneally) administration route. The

pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Pharmaceutical compositions according to the invention may be formulated to release the active compound substantially immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the anti-parasitic within the body over an extended period of time; (ii) formulations that after a predetermined lag time create a substantially constant concentration of the anti-parasitic within the body over an extended period of time; (iii) formulations that sustain anti-parasitic action during a predetermined time period by maintaining a relatively, constant, effective anti-parasitic level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active anti-parasitic substance (sawtooth kinetic pattern); (iv) formulations that localize anti-parasitic action by, e.g., spatial placement of a controlled release composition adjacent to or in the infected tissue or organ; (v) formulations that allow for convenient dosing, such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target a parasite by using carriers or chemical derivatives to deliver the anti-parasitic to a particular parasite or parasite infected cell type. Administration of anti-parasitic compounds in the form of a controlled release formulation is especially preferred for anti-parasitics having a narrow absorption window in the gastro-intestinal tract or a very short biological half-life. In these cases, controlled release formulations obviate the need for frequent dosing during the day to sustain the plasma level at a therapeutic level.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the anti-parasitic is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the anti-parasitic in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

Parenteral Compositions

The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation. Formulations can be found in Remington: The Science and Practice of Pharmacy, *supra*.

Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active anti-parasitic (s), the composition may include suitable parenterally acceptable carriers and/or excipients. The active anti-parasitic (s) may be incorporated into microspheres, microcapsules, nanoparticles, liposomes, or the

like for controlled release. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing agents.

As indicated above, the pharmaceutical compositions according to the invention may be in the form suitable for sterile injection. To prepare such a composition, the suitable active anti-parasitic (s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, and isotonic sodium chloride solution and dextrose solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

Controlled Release Parenteral Compositions

Controlled release parenteral compositions may be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, or emulsions. Alternatively, the active anti-parasitic (s) may be incorporated in biocompatible carriers, liposomes, nanoparticles, implants, or infusion devices.

Materials for use in the preparation of microspheres and/or microcapsules are, e.g., biodegradable/bioerodible polymers such as polygalactin, poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutamnine) and, poly(lactic acid). Biocompatible carriers that may be used when formulating a controlled release parenteral formulation are carbohydrates (e.g., dextrans), proteins (e.g., albumin), lipoproteins, or antibodies.

Materials for use in implants can be non-biodegradable (e.g., polydimethyl siloxane) or biodegradable (e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters) or combinations thereof).

Solid Dosage Forms For Oral Use

Formulations for oral use of interferon include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. Such formulations are known to the skilled artisan. Excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the active anti-parasitic substance in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the active anti-parasitic substance until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl

methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose). Furthermore, a time delay material such as, e.g., glyceryl monostearate or glyceryl distearate may be employed.

The solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active anti-parasitic substance). The coating may be applied on the solid dosage form in a similar manner as that described in Encyclopedia of Pharmaceutical Technology, supra.

The two anti-parasitics may be mixed together in the tablet, or may be partitioned. In one example, the first anti-parasitic is contained on the inside of the tablet, and the second anti-parasitic is on the outside, such that a substantial portion of the second anti-parasitic is released prior to the release of the first anti-parasitic .

Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Controlled Release Oral Dosage Forms

Controlled release compositions for oral use may, e.g., be constructed to release the active anti-parasitic by controlling the dissolution and/or the diffusion of the active anti-parasitic substance.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated metylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

A controlled release composition containing one or more of the compounds of the claimed combinations may also be in the form of a buoyant tablet or capsule (i.e., a tablet or capsule that, upon oral administration, floats on top of the gastric content for a certain period of time). A buoyant tablet formulation of the compound(s) can be prepared by granulating a mixture of the anti-parasitic (s) with excipients and 20-75% w/w of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose, or hydroxypropylmethylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet forms a substantially

water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of less than one, thereby allowing the tablet to remain buoyant in the gastric juice.

Combination Therapies

Anti-parasitics may be administered in combination with any other standard anti-parasitic therapy; such methods are known to the skilled artisan and described in Remington's Pharmaceutical Sciences by E.W. Martin.

Insecticides and Nematicides

Insecticides and nematicides are also provided by the methods described herein to control insects and nematodes. Such insecticides and nematicides are expected to be superior to existing insecticides and nematicides: (i) because they are specific to insect or nematode proteins and therefore unlikely to have adverse effects on humans; (ii) because they arrest development during molting, a non-feeding stage, in contrast to juvenile hormone insecticides which arrest development during a feeding stage; and/or (iii) because they result in an agriculturally desirable insect kill or "knockdown." Methods for the production and application of insecticides or nematicides are standard in the art and described herein.

A method of controlling an insect, nematode, or other Ecdysozoan population is provided by the invention. The method involves contacting an insect or nematode with a biocidally effective amount of a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. Such methods may be used to kill or reduce the numbers of insects or nematodes in a given area, or may be prophylactically applied to an area to prevent infestation by a susceptible Ecdysozoan. Preferably the insect or nematode ingests, or is contacted with, an biocidally-effective amount of the MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor.

Insect Pests

Virtually all field crops, plants, and commercial farming areas are susceptible to attack by one or more insect pests. Such insect pests may be targeted with an insecticide containing a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. For example, vegetable and cole crops such as artichokes, kohlrabi, arugula, leeks, asparagus, lentils, beans, lettuce (e.g. head, leaf, romaine), beets, bok choy, malanga, broccoli, melons (e.g., muskmelon, watermelon, crenshaw, honeydew, cantaloupe), brussels sprouts, cabbage, cardoni, carrots, napa, cauliflower, okra, onions, celery, parsley, chick peas, parsnips, chicory, peas, Chinese cabbage, peppers, collards, potatoes, cucumber, pumpkins, cucurbits, radishes, dry bulb onions, rutabaga, eggplant, salsify, escarole, shallots, endive, soybean, garlic, spinach, green onions, squash, greens, sugar beets, sweet potatoes, turnip, swiss chard, horseradish, tomatoes, kale, turnips, and a variety of spices are sensitive to infestation by one or more of the following insect pests: alfalfa looper, armyworm, beet armyworm, artichoke plume moth, cabbage budworm, cabbage looper, cabbage webworm, corn earworm, celery leafeater, cross-striped cabbageworm, european corn borer, diamondback moth, green cloverworm, imported cabbageworm, melonworm, omnivorous leafroller, pickleworm, rindworm complex, saltmarsh caterpillar, soybean looper, tobacco budworm, tomato fruitworm, tomato hornworm, tomato pinworm, velvetbean caterpillar, and yellowstriped armyworm.

Likewise, pasture and hay crops such as alfalfa, pasture grasses and silage are often attacked by such pests as armyworm, beef armyworm, alfalfa caterpillar, European skipper, a variety of loopers and webworms, as well as yellowstriped armyworms.

Fruit and vine crops such as apples, apricots, cherries, nectarines, peaches, pears, plums, prunes, quince almonds, chestnuts, filberts, pecans, pistachios, walnuts, citrus, blackberries, blueberries, boysenberries, cranberries, currants, loganberries, raspberries, strawberries, grapes, avocados, bananas,

kiwi, persimmons, pomegranate, pineapple, tropical fruits are often susceptible to attack and defoliation by achema sphinx moth, amorbia, armyworm, citrus cutworm, banana skipper, blackheaded fireworm, blueberry leafroller, cankerworm, cherry fruitworm, citrus cutworm, cranberry girdler, eastern tent caterpillar, fall webworm, fall webworm, filbert leafroller, filbert webworm, fruit tree leafroller, grape berry moth, grape leaffolder, grapeleaf skeletonizer, green fruitworm, gummosos-batrachedra commosae, gypsy moth, hickory shuckworm, hornworms, loopers, navel orangeworm, obliquebanded leafroller, omnivorous leafroller. omnivorous looper, orange tortrix, orangedog, oriental fruit moth, pandemis leafroller, peach twig borer, pecan nut casebearer, redbanded leafroller, redhumped caterpillar, rougliskinned cutworm, saltmarsh caterpillar, spanworm, tent caterpillar, thecla-thecla basillides, tobacco budworm, tortrix moth, tufted apple budmoth, variegated leafroller, walnut caterpillar, western tent caterpillar, and yellowstriped armyworm.

Field crops such as canola/rape seed, evening primrose, meadow foam, corn (field, sweet, popcorn), cotton, hops, jojoba, peanuts, rice, safflower, small grains (barley, oats, rye, wheat, etc.), sorghum, soybeans, sunflowers, and tobacco are often targets for infestation by insects including armyworm, asian and other corn borers, banded sunflower moth, beet armyworm, bollworm, cabbage looper, corn rootworm (including southern and western varieties), cotton leaf perforator, diamondback moth, european corn borer, green cloverworm, headmoth, headworm, imported cabbageworm, loopers (including Anacamptodes spp.), obliquebanded leafroller, omnivorous leaftier, podworm, podworm, saltmarsh caterpillar, southwestern corn borer, soybean looper, spotted cutworm, sunflower moth, tobacco budworm, tobacco hornworm, velvetbean caterpillar,

Bedding plants, flowers, ornamentals, vegetables and container stock are frequently fed upon by a host of insect pests such as armyworm, azalea moth,

beet armyworm, diamondback moth, ello moth (hornworm), Florida fern caterpillar, Io moth, loopers, oleander moth, omnivorous leafroller, omnivorous looper, and tobacco budworm.

Forests, fruit, ornamental, and nut-bearing trees, as well as shrubs and other nursery stock are often susceptible to attack from diverse insects such as bagworm, blackheaded budworm, browntail moth, California oakworm, douglas fir tussock moth, elm spanworm, fall webworm, fuittree leafroller, greenstriped mapleworm, gypsy moth, jack pine budworm, mimosa webworm, pine butterfly, redhumped caterpillar, saddleback caterpillar, saddle prominent caterpillar, spring and fall cankerworm, spruce budworm, tent caterpillar, tortrix, and western tussock moth. Likewise, turf grasses are often attacked by pests such as armyworm, sod webworm, and tropical sod webworm.

Nematode Agricultural Pests

Virtually all field crops, plants, and commercial farming areas are susceptible to attack by one or more nematode pests. Examples of plants subject to nematode attack include, but are not limited to, rice, wheat, maize, cotton, potato, sugarcane, grapevines, cassava, sweet potato, tobacco, soybean, sugar beet, beans, banana, tomato, lettuce, oilseed rape and sunflowers. Nematodes to be controlled using a nematicide containing a *mlt* nucleic acid or MLT polypeptide include, but are not limited to, plant parasites belonging to the Orders Dorylaimida and Tylenchida. Nematodes which may be controlled by this invention include, but are not limited to Families Longidoridae (e.g., *Xiphinema* spp. and *Longidorus* spp.) or Trichodoridae, (e.g., *Trichodorus* spp. and *Paratrichodorus* spp), migratory ectoparasites belonging to the Families Anguinidae (e.g., *Ditylenchus* spp.), Dolichodoridae (*Dolichodorus* spp.) and Belenolaimidae (e.g., *Belenolaimus* spp. and *Trophanus* spp).; obligate parasites belonging to the -Families Pratylenchidae (e.g., *Pratylenchus* spp., *Radopholus* spp. and *Nacobbus* spp), Hoplolaimidae (e.g., Helicotylenchus spp., Scutellonema spp. and Rotylenchulus spp.), Heteroderidae (e.g.,

Heterodera spp., *Globodera* spp., *Meloidogyne* spp. and *Meloinema* spp.), Criconematidae (e.g., *Croconema* spp., *Criconemella* spp. *Hemicycliophora* spp.), and Tylenchulidae (e.g., *Tylenchulus* spp., *Paratylenchulus* spp. and Tylenchocriconema spp.); and parasites belonging to the Families Aphelenchoididae (e.g., *Aphelenchoides* spp., *Bursaphelenchus* spp. and *Rhadinaphelenchus* spp.) and Fergusobiidae (e.g., *Fergusobia* spp.).

Insecticidal or Nematicidal Compositions and Methods of Use

In one preferred embodiment, the MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor compositions disclosed herein are useful as insecticides or nematicides for topical and/or systemic application to field crops, grasses, fruits and vegetables, lawns, trees, and/or ornamental plants. Alternatively, a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor disclosed herein may be formulated as a spray, dust, powder, or other aqueous, atomized or aerosol for killing an Ecdysozoan (e.g., an insect, or nematode) or controlling an Ecdysozoan population. The MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor compositions disclosed herein may be used prophylactically, or alternatively, may be administered to an environment once target Ecdysozoans have been identified in the particular environment to be treated.

Regardless of the method of application, the amount of the active polypeptide component(s) is applied at a biocidally-effective amount, which will vary depending on such factors as, for example, the specific target Ecdysozoan to be controlled, the specific environment, location, plant, crop, or agricultural site to be treated, the environmental conditions, and the method, rate, concentration, stability, and quantity of application of the biocidally-active polypeptide composition. The formulations may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of insect infestation.

The insecticide and nematicide compositions described may be made by formulating the isolated MLT protein with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material, a suspension in oil (vegetable or mineral), water, or oil/water emulsion, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. An agriculturally-acceptable carrier includes but is not limited to, for example, adjuvants, inert components, dispersants, surfactants, tackifiers, and binders, that are ordinarily used in insecticide or nematicide formulation technology. Such carriers are well known to those skilled in insecticide or nematicide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g., by homogeneously mixing, blending and/or grinding the insecticidal composition with suitable adjuvants using conventional formulation techniques.

Oil Flowable Suspensions

In a preferred embodiment, the insecticide or nematicide composition comprises an oil flowable suspension comprising a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor, or bacterial cell expressing a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. In one preferred embodiment, the bacterial cells are *B. thuringiensis* or *E. coli,* but any bacterial host cell expressing aMLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor may be useful. Exemplary bacterial species include *B. thuringiensis, B. megaterium, B. subtilis, B. cereus, E. coli, Salmonella spp., Agrobacterium spp., or Pseudomonas spp.*

Water-Dispersible Granules

In another important embodiment, the insecticide composition comprises a water dispersible granule. This granule comprises a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor, or bacterial cell expressing a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. In one preferred embodiment, the bacterial cells are *B. thuringiensis* or *E. coli*, but other bacteria such as *B. megaterium, B. subtilis, B. cereus, E. coli, Salmonella spp., Agrobacterium spp.*, or *Pseudomonas spp.* cells transformed with a DNA segment disclosed herein and expressing a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor are also contemplated to be useful.

Powders, Dusts, and Spore Formulations

For some applications, the insecticide composition comprises a wettable powder, dust, spore crystal formulation, cell pellet, or colloidal concentrate. This powder comprises a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor, or a bacterial cell expressing a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. Preferred bacterial cells are *B. thuringiensis* or *E. coli*, however, bacterial cells such as those of other strains of *B. thuringiensis*, or cells of strains of bacteria such as *B. megaterium, B. subtilis, B. cereus, E. coli, Salmonella spp., Agrobacterium spp.*, or *Pseudomonas spp.*, may also be transformed with one or more *mlt* nucleic acid. Such dry forms of the insecticidal compositions may be formulated to dissolve immediately upon wetting, or alternatively, dissolve in a controlled-release, sustained-release, or other time-dependent manner. Such compositions may be applied to, or ingested by, the target insect, and as such, may be used to control the numbers of insects, or the spread of such insects in a given environment.

Aqueous Suspensions and Bacterial Cell Filtrates or Lysates

For some applications, the insecticide or nematicide composition comprises an aqueous suspension of bacterial cells, or an aqueous suspension of bacterial cell lysates or filtrates, etc., containing a MLT polypeptide, *mlt*

nucleic acid, or RNA *mlt* nucleic acid inhibitor. Such aqueous suspensions may be provided as a concentrated stock solution which is diluted prior to application, or alternatively, as a diluted solution ready-to-apply.

The insecticidal or nematicidal compositions comprise intact bacterial cells expressing a *mlt* nucleic acid or polypeptide. These compositions may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

Alternatively, the novel insecticidal or nematicidal polypeptides may be prepared by native or recombinant bacterial expression systems in vitro and isolated for subsequent field application. Such protein may be either in crude cell lysates, suspensions, colloids, etc., or alternatively may be purified, refined, buffered, and/or further processed, before formulating in an active biocidal formulation. Likewise, under certain circumstances, it may be desirable to isolate a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor from the bacterial cultures expressing the MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor and apply solutions, suspensions, or colloidal preparations of such nucleic acids or proteins as the active bioinsecticidal composition.

Multifunctional Formulations

In some embodiments, when the control of multiple Ecdysozoan species is desired, the insecticidal or nematicidal formulations described herein may comprise one or more chemical pesticides, (such as chemical pesticides, nematicides, fungicides, virucides, microbicides, amoebicides, insecticides,

etc.), and/or one or MLT polypeptides, *mlt* nucleic acids, or RNA *mlt* nucleic acid inhibitors. The insecticidal polypeptides may also be used in conjunction with other treatments such as fertilizers, weed killers, cryoprotectants, surfactants, detergents, insecticidal soaps, dormant oils, polymers, and/or time-release or biodegradable carrier formulations that permit long-term dosing of a target area following a single application of the formulation. In addition, the formulations may be prepared in edible baits or fashioned into insect or nematode traps to permit feeding or ingestion by a target Ecdysozoan of the biocide formulation.

The insecticidal compositions of the invention may also be used in consecutive or simultaneous application to an environmental site singly or in combination with one or more additional insecticides, pesticides, chemicals, fertilizers, or other compounds.

Application Methods and Effective Rates

The insecticidal or nematicidal compositions of the invention are applied to the environment of the target Ecdysozoan, typically onto the foliage of the plant or crop to be protected, by conventional methods, preferably by spraying. The strength and duration of application will be set with regard to conditions specific to the particular pest(s), crop(s) to be treated and particular environmental conditions. The proportional ratio of active ingredient to carrier will naturally depend on the chemical nature, solubility, and stability of the insecticidal composition.

Other application techniques, including dusting, sprinkling, soil soaking, soil injection, seed coating, seedling coating, foliar spraying, aerating, misting, atomizing, fumigating, aerosolizing, and the like, are also feasible and may be required under certain circumstances such as e.g., insects that cause root or stalk infestation, or for application to delicate vegetation or ornamental plants. These application procedures are also well-known to those of skill in the art.

The insecticidal or nematicidal compositions of the present invention may also be formulated for preventative or prophylactic application to an area, and may in certain circumstances be applied to pets, livestock, animal bedding, or in and around farm equipment, barns, domiciles, or agricultural or industrial facilities, and the like.

The concentration of an insecticidal or nematicidal composition that is used for environmental, systemic, topical, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of biocidal activity. Typically, the biocidal, insecticidal, or nematicidal composition will be present in the applied formulation at a concentration of at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% by weight. Dry formulations of MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor compositions may be from about 1% to about 99% or more by weight of the nucleic acid or polypeptide composition, while liquid formulations may generally comprise from about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99% or more of the active ingredient by weight.

In the case of compositions in which intact bacterial cells that contain at least one MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor are included, preparations will generally contain from about 10^4 to about 10^8 cells/mg, although in certain embodiments it may be desirable to utilize formulations comprising from about 10^2 to about 10^4 cells/mg, or when more concentrated formulations are desired, compositions comprising from about 10^8 to about 10^{10} or 10^{11} cells/mg may also be formulated. Alternatively, cell pastes, spore concentrates, MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor concentrates may be prepared that contain the equivalent of from about 10^{12} to 10^{13} cells/mg of the active polypeptide, and such concentrates may be diluted prior to application.

The insecticidal or nematicidal formulation described above may be administered to a particular plant or target area in one or more applications as needed, with a typical field application rate per hectare ranging on the order of about 50, 100, 200, 300, 400, or 500 g/hectare of active ingredient, or alternatively, 600, 700, 800, 900, or 1000 g/hectare may be utilized. In certain instances, it may even be desirable to apply the insecticidal or nematicidal formulation to a target area at an application rate of about 1000, 2000, 3000, 4000, 5000 g/hectare or even as much as 7500, 10,000, or 15,000 g/hectare of active ingredient.

MLT Polypeptide Insecticides and Nematicides

As discussed above, MLT polypeptide, *mlt* nucleic acid, and RNA *mlt* nucleic acid inhibitor are useful, for example, for inhibiting molting in an Ecdysozoan (e.g., a parasitic insect or nematode). Such nucleic acids and polypeptides may be, for example, applied ectopically or administered systemically to a plant at a level that is sufficient to inhibit insect or nematode infestation in the plant. Evaluation of the level of insect or nematode protection conferred to a plant by application or administration of a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor is determined according to conventional methods and assays.

In one embodiment, a plant is contacted with a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor present in an excipient, such that a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor is present in or on the plant (e.g., in or on the roots, leaves, stems, fruit, flowers, or vegetative tissues). A parasitic insect or nematode is introduced to the plant under controlled conditions (for example, standard levels of temperature, humidity, and/or soil conditions). After a period of incubation sufficient to allow the growth and reproduction of a harmful insect or nematode on a control plant not contacted with a MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor, insects, nematodes, or their progeny are evaluated for

their level of growth, viability, or reproduction according to conventional experimental methods. For example, the number of insects, nematodes, or their progeny is recorded every twenty-four hours for seven days, fourteen days, twenty-one days, or twenty-eight days or longer after inoculation. From these data, levels of inhibition of harmful insects or nematodes are determined. MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitors that inhibit the growth, viability, or reproduction of a harmful insect or nematode are taken as being useful in the invention. In another embodiment, the level of plant damage is determined according to standard methods on the plant contacted with the MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor relative to a control plant not contacted with the MLT polypeptide, *mlt* nucleic acid, or RNA *mlt* nucleic acid inhibitor. MLT polypeptides, *mlt* nucleic acids, or RNA *mlt* nucleic acid inhibitors that inhibit plant damage are taken to be useful in the methods of the invention.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adapt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication was specifically and individually indicated to be incorporated by reference.

What is claimed is: